

| | L # | Hits | Search Text | DBs | Time Stamp |
|---|-----|------|--|--------------------|---------------------|
| 1 | L1 | 814 | fructosyltransferase\$1 or fructosyl adj transferase\$1 or \$sucrase | USPAT; US-PGPUB | 2002/10/08 11:59 |
| 2 | L2 | 14 | 1 same (lactobacillus or lactic adj acid adj bacteri\$8) | USPAT; US-PGPUB | 2002/10/08 12:01 |
| 3 | L3 | 109 | 1 same (fructan or levan or inulin) | USPAT; US-PGPUB | 2002/10/08 14:10 |
| 4 | L4 | 83 | 3 same (mak\$6 or produc\$8 or synthes\$8) | USPAT; US-PGPUB | 2002/10/08 14:09 |
| 5 | L5 | 62 | 1 same ((fructan or levan or inulin) near5 (mak\$6 or produc\$8 or synthes\$8)) | USPAT; US-PGPUB | 2002/10/08 14:11 |
| 6 | L6 | 72 | 2 or 5 | USPAT; US-PGPUB | 2002/10/08 14:11 |

PGPUB-DOCUMENT-NUMBER: 20020127681

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020127681 A1

TITLE: Novel fructosyltransferases

PUBLICATION-DATE: September 12, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|--------------------|-------|---------|---------|
| Van Hijum, Sacha | Groningen | | NL | |
| Adrianus Fokke Taco | Driebergen-Rijsend | | NL | |
| Van Geel-Schutten, | berg | | NL | |
| Gerritdina Hendrika | Zuidlaren | | NL | |
| Dijkhuizen, Lubbert | Amersfoort | | | |
| Rahaoui, Hakim | | | | |

APPL-NO: 09/ 995587

DATE FILED: November 29, 2001

RELATED-US-APPL-DATA:

child 09995587 A1 20011129 parent continuation-in-part-of 09604958 20000628 US
PENDING

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|------------|-------------------|--------------|
| EP | 00201872.9 | 2000EP-00201872.9 | May 25, 2000 |

US-CL-CURRENT: 435/193,435/101 ,435/252.3 ,435/325 ,435/69.1 ,536/123
,536/23.2

ABSTRACT:

The present invention describes two novel proteins having fructosyltransferase activity. Both enzymes are derived from lactobacilli, which are food-grade micro-organisms with the Generally Recognized As Safe (GRAS) status. One of these proteins produces an inulin and fructo-oligosaccharides, while the other produces a levan and fructo-oligosaccharides. According to the invention lactobacilli capable of producing an inulin and/or a levan and/or fructo-oligosaccharides using one or both of the fructosyltransferases can be used as a probiotic or a symbiotic.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation-in-part application of U.S. application Ser. No. 09/604,958 filed on Jun. 28, 2000, which claims priority

from European Application No. 00201872.9 filed on May 25, 2000.

----- KWIC -----

Abstract Paragraph - ABTX:

The present invention describes two novel proteins having fructosyltransferase activity. Both enzymes are derived from lactobacilli, which are food-grade micro-organisms with the Generally Recognized As Safe (GRAS) status. One of these proteins produces an inulin and fructo-oligosaccharides, while the other produces a levan and fructo-oligosaccharides. According to the invention lactobacilli capable of producing an inulin and/or a levan and/or fructo-oligosaccharides using one or both of the fructosyltransferases can be used as a probiotic or a symbiotic.

Summary of Invention Paragraph - BSTX:

[0006] The exopolysaccharides produced by LAB can be divided in two groups, heteropolysaccharides and homopolysaccharides; these are synthesized by totally different mechanisms. The former consist of repeating units in which residues of different types of sugars are present and the latter consist of one type of monosaccharide. The synthesis of heteropolysaccharides by lactic acid bacteria, including lactobacilli, has been studied extensively in recent years. Considerably less information is available on the synthesis of homopolysaccharides from lactobacilli, although some studies have been performed. Homopolysaccharides with fructose as the constituent sugar can be divided into two groups, inulins and levans. Inulins consist of 2,1-linked .beta.-fructofuranoside residues, whereas levans consist of 2,6-linked .beta.-fructofuranoside residues. Both can be linear or branched. The size of bacterial levans can vary from 20 kDa up to several MDa. There is limited information on the synthesis of levans. In most detail this synthesis has been studied in *Zymomonas mobilis* and in *Bacillus* species. Within lactic acid bacteria, fructosyltransferases have only been studied in streptococci. So far no fructosyltransferases have been reported in lactobacilli.

Summary of Invention Paragraph - BSTX:

[0008] Two novel genes encoding enzymes having fructosyltransferase activity have now been found in Lactobacillus reuteri, and their amino acid sequences have been determined. These are the first two enzymes identified in a Lactobacillus species capable of producing a fructan. One of the enzymes is an inulosucrase which produces a high molecular weight (>10⁷ Da) fructan containing .beta.(2-1) linked fructosyl units and fructo-oligosaccharides, while the other is a levansucrase which produces a fructan containing .beta.(2-6) linked fructosyl units. The invention thus pertains to the enzymes, to DNA encoding them, to recombinant cells containing such DNA and to their use in producing carbohydrates, as defined in the appending claims.

Summary of Invention Paragraph - BSTX:

[0009] It was found according to the invention that one of the novel **fructosyltransferases** (FTFA; an **inulosucrase**) **produces a high molecular weight inulin** with .beta.(2-1) linked fructosyl units and fructo-oligosaccharides. The fructo-oligosaccharides synthesis was also observed in certain **Lactobacillus** strains, in particular in certain strains of **Lactobacillus reuteri**. However, the inulin has not been found in **Lactobacillus reuteri** culture supernatants, but only in extracts of E. coli cells expressing the above-mentioned **fructosyltransferase**. This **inulosucrase** consists of either 798 amino acids (2394 nucleotides) or 789 amino acids (2367 nucleotides) depending on the potential start codon used. The molecular weight (MW) deduced of the amino acid sequence of the latter form is 86 kDa and its isoelectric point is 4.51, at pH 7.

Summary of Invention Paragraph - BSTX:

[0012] **Fructosyltransferases** have been found in several bacteria such as Zymomonas mobilis, Erwinia amylovora, Acetobacter amylovora, Bacillus polymyxa, Bacillus amyloliquefaciens, Bacillus stearothermophilus, and Bacillus subtilis. In **lactic acid bacteria** this type of enzyme previously has only been found in some streptococci. Most bacterial **fructosyltransferases** have a molecular mass of 50-100 kDa (with the exception of the **fructosyltransferase** found in Streptococcus salivarius which has a molecular mass of 140 kDa). Amino acid sequence alignment revealed that the novel **inulosucrase** of lactobacilli has high homology with **fructosyltransferases** originating from Gram positive bacteria, in particular with Streptococcus enzymes. The highest homology (FIG. 2) was found with the SacB enzyme of Streptococcus mutans Ingbritt A (62% identity within 539 amino acids).

Summary of Invention Paragraph - BSTX:

[0015] A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The **inulosucrase** gene (starting at nucleotide 41) has been cloned in an E. coli expression vector under the control of an ara promoter in E. coli Top10. E. coli Top10 cells expressing the recombinant **inulosucrase** hydrolysed sucrose and **synthesized fructan** material. SDS-PAGE of arabinose induced E. coli Top10 cell extracts suggested that the recombinant **inulosucrase** has a molecular weight of 80-100 kDa, which is in the range of other known **fructosyltransferases** and in line with the molecular weight of 86 kDa deduced of the amino acid sequence depicted in FIG. 1.

Summary of Invention Paragraph - BSTX:

[0016] The invention further covers an **inulosucrase** according to the invention which, in the presence of sucrose, **produces a inulin** having .beta.(2-1)-linked

D-fructosyl units and fructo-oligosaccharides. Two different types of fructans, inulins and levans, exist in nature. Surprisingly, the novel inulosucrase expressed in E. coli Top10 cell synthesizes a high molecular weight (>10⁵ Da) inulin and fructo-oligosaccharides, while in Lactobacillus reuteri culture supernatants, in addition to the fructo-oligosaccharides, a levan and not an inulin is found. This discrepancy can have several explanations: the inulosucrase gene may be silent in Lactobacillus reuteri, or may not be expressed in Lactobacillus reuteri under the conditions tested, or the inulosucrase may only synthesize fructo-oligosaccharides in its natural host, or the inulin polymer may be degraded shortly after synthesis, or may not be secreted and remains cell-associated, or the inulosucrase may have different activities in Lactobacillus reuteri and E. coli Top10 cells.

Summary of Invention Paragraph - BSTX:

[0017] It was furthermore found according to the invention that certain lactobacilli, in particular Lactobacillus reuteri, possess another fructosyltransferase, a levansucrase (FTFB), in addition to the inulosucrase described above. The N-terminal amino acid sequence of the fructosyltransferase purified from Lactobacillus reuteri supernatant was found to be QVESNNYNGVAEVNTERQANGQI (SEQ ID No. 6). Furthermore, three internal sequences were identified, namely (M)(A)HLDVWDSWPVQDP(V) (SEQ ID No. 7), NAGSIFGT(K) (SEQ ID No. 8), V(E)(E)VYSPKVSTLMASDEVE (SEQ ID No. 9). The N-terminal amino acid sequence could not be identified in the deduced inulosucrase sequence. Also the amino acid sequences of the three internal peptide fragments of the purified fructosyltransferase were not present in the putative inulosucrase sequence. Evidently, the inulosucrase gene does not encode the purified fructosyltransferase synthesizing the levan. The complete amino acid sequence of the levansucrase is shown in SEQ ID No. 11 and the nucleotide sequence is shown in SEQ ID No. 10. The levansucrase comprises a putative membrane anchor (see amino acids 761-765 in SEQ ID No. 11) and a putative membrane spanning domain (see amino acids 766-787 in SEQ ID No. 11). The fructan produced by the levansucrase was identified in the Lactobacillus reuteri culture supernatant as a linear (2,6)- β -D-fructofuranan with a molecular weight of 150 kDa. The purified enzyme also produces this fructan.

Summary of Invention Paragraph - BSTX:

[0018] Additionally, the invention thus covers a protein having levansucrase activity with an amino acid identity of at least 65%, preferably at least 75%, and more preferably at least 85%, compared to the amino acid sequence of SEQ ID NO. 11. The second novel fructosyltransferase produces a high molecular weight fructan with (2-6) linked fructosyl units with sucrose or raffinose as substrate. The invention also covers a part of a protein with least 15 contiguous amino acids, which are identical to the corresponding part of the amino acid sequence of SEQ ID No. 11. A nucleotide sequence encoding any of the above-mentioned proteins, mutants, variants or parts thereof is a subject of the invention as well as a nucleic acid construct comprising the nucleotide

sequence mentioned above operationally linked to an expression-regulating nucleic acid sequence. A recombinant host cell, such as a mammalian (with the exception of human), plant, animal, fungal or bacterial cell, containing one or more copies of the nucleic acid construct mentioned above is an additional subject of the invention. The invention further covers a protein according to the invention which, in the presence of sucrose, **produces a fructan** having .beta.(2-6)-linked D-fructosyl units.

Summary of Invention Paragraph - BSTX:

[0019] The invention also pertains to a process of **producing an inulin-type** and/or a levan-type of fructan as described above using **fructosyltransferases** according to the invention and a suitable fructose source such as sucrose, stachyose or raffinose. The fructans may either be produced by **Lactobacillus** strains or recombinant host cells according to the invention containing one or both **fructosyl transferases** or by a fructosyltransferase enzyme isolated by conventional means from the culture of **fructosyltransferase**-positive lactobacilli, especially a **Lactobacillus** reuteri, or from a recombinant organism containing the **fructosyltransferase** gene or genes.

Summary of Invention Paragraph - BSTX:

[0020] Additionally, the invention concerns a process of producing fructo-oligosaccharides containing the characteristic structure of the fructans described above using a **Lactobacillus** strain or a recombinant host cell according to the invention containing one or both **fructosyltransferases** or an isolated **fructosyltransferase** according to the invention. There is a growing interest in oligosaccharides derived from homopolysaccharides, for instance for prebiotic purposes. Several fructo- and gluco-oligosaccharides are known to stimulate the growth of bifidobacteria in the human colon. Fructo-oligosaccharides produced by the **fructosyltransferase** described above are also part of the invention. Another way of producing fructo-oligosaccharides is by hydrolysis of the fructans described above. This hydrolysis can be performed by known hydrolysis methods such as enzymatic hydrolysis with enzymes such as levanase or inulinase or by acid hydrolysis. The fructo-oligosaccharides can also be produced in the presence of a **fructosyltransferase** according to the invention and an acceptor molecule such as lactose or maltose. The fructo-oligosaccharides to be produced according to the invention preferably contain at least 2, more preferably at least 3, up to about 20 anhydrofructose units, optionally in addition to one or more other (glucose, galactose, etc.) units. These fructo-oligosaccharides are useful as prebiotics, and can be administered to a mammal in need of improving the bacterial status of the colon..

Summary of Invention Paragraph - BSTX:

[0024] Use of a **Lactobacillus** strain capable of **producing a levan**, inulin or fructo-oligosaccharides or a mixture thereof, as a probiotic, is also covered by the invention. Preferably, the **Lactobacillus** strain is also capable of

producing a glucan, especially an 1,4/1,6- α -glucan as referred to above. The efficacy of some Lactobacillus reuteri strains as a probiotics has been demonstrated in various animals such as for instance poultry and humans. The administration of some Lactobacillus reuteri strains to pigs resulted in significantly lower serum total and LDL-cholesterol levels, while in children Lactobacillus reuteri is used as a therapeutic agent against acute diarrhea. For this and other reasons Lactobacillus reuteri strains, which were not reported to produce the glucans or fructans described herein, have been supplemented to commercially available probiotic products. The mode of action of Lactobacillus reuteri as a probiotic is still unclear. Preliminary studies indicated that gut colonization by Lactobacillus reuteri may be of importance. According to the invention, it was found that the mode of action of Lactobacillus reuteri as a probiotic may reside partly in the ability to produce polysaccharides. Lactobacillus strains, preferably Lactobacillus reuteri strains, and more preferably Lactobacillus reuteri strain LB 121 and other strains containing one or more fructosyltransferase genes encoding proteins capable of producing inulins, levans and/or fructo-oligosaccharides can thus advantageously be used as a probiotic. They can also, together with these polysaccharides, be used as a symbiotic (instead of the term symbiotic, the term synbiotic can also be used). In that respect another part of the invention concerns a probiotic or symbiotic composition containing a Lactobacillus strain capable of producing an inulin, a levan or fructo-oligosaccharides and/or a glucan or a mixture thereof, said production being performed according to the process according to the invention. The probiotic or symbiotic compositions of the invention may be directly ingested with or without a suitable vehicle or used as an additive in conjunction with foods. They can be incorporated into a variety of foods and beverages including, but not limited to, yoghurts, ice creams, cheeses, baked products such as bread, biscuits and cakes, dairy and dairy substitute foods, confectionery products, edible oil compositions, spreads, breakfast cereals, juices and the like.

Detail Description Paragraph - DETX:

[0026] Isolation of DNA from Lactobacillus reuteri Nucleotide Sequence Analysis of the Inulosucrase (ftfA) Gene, Construction of Plasmids for Expression of the Inulosucrase Gene in E. coli Top10 Expression of the Inulosucrase gene in E. coli Top10 and Identification of the Produced Polysaccharides Produced by the Recombinant Enzyme.

Detail Description Paragraph - DETX:

[0027] General procedures for cloning, DNA manipulations and agarose gel electrophoresis were essentially as described by Sambrook et al. (1989) Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York. Restriction endonuclease digestions and ligations with T4 DNA ligase were performed as recommended by the suppliers. DNA was amplified by PCR techniques using ampliTAQ DNA polymerase (Perkin Elmer) or Pwo DNA polymerase. DNA fragments were isolated from agarose gels using the Qiagen extraction kit (Qiagen GMBH), following the instructions of

the suppliers. Lactobacillus reuteri strain 121 (LMG 18388) was grown at 37.degree. C. in MRS medium (DIFCO) or in MRS-s medium (MRS medium containing 100 g/l sucrose instead of 20 g/l glucose). When fructo-oligosaccharides production was investigated phosphate was omitted and ammonium citrate was replaced by ammonium nitrate in the MRS-s medium. E. coli strains were grown aerobically at 37.degree. C. in LB medium, where appropriate supplemented with 50 .mu.g/ml ampicillin (for selection of recombinant plasmids) or with 0.02% (w/v) arabinose (for induction of the inulosucrase gene).

Detail Description Paragraph - DETX:

[0029] The inulosucrase gene was identified by amplification of chromosomal DNA of Lactobacillus reuteri with PCR using degenerated primers (5ftf, 6ftfi, and 12ftfi, see table 1) based on conserved amino acid sequences deduced from different bacterial fructosyltransferase genes (SacB of Bacillus amyloliquefaciens, SacB of Bacillus subtilis, Streptococcus mutans fructosyltransferase and Streptococcus salivarius fructosyltransferase, see FIG. 4) and Lactobacillus reuteri DNA as template. Using primers 5ftf and 6ftfi, an amplification product with the predicted size of about 234 bp was obtained (FIG. 5A). This 234 bp fragment was cloned in E. coli JM109 using the pCR2.1 vector and sequenced. Transformations were performed by electroporation using the BioRad gene pulser apparatus at 2.5 kV, 25 .mu.F and 200 .OMEGA., following the instructions of the manufacturer. Sequencing was performed according to the method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467. Analysis of the obtained sequence data confirmed that part of a fructosyltransferase (ftf gene had been isolated. The 234 bp amplified fragment was used to design primers 7ftf and 8ftfi (see table 1). PCR with the primers 7ftf and 12ftfi gave a product of the predicted size of 948 bp (see FIG. 5B); its sequence showed clear similarity with previously characterized fructosyltransferase genes. The 948 bp amplified fragment was used to design the primers ftfAC1(i) and ftfAC2(i) (see table 1) for inverse PCR. Using inverse PCR techniques a 1438 bp fragment of the inulosucrase gene was generated, including the 3' end of the inulosucrase gene (see FIG. 5C). The remaining 5' fragment of the inulosucrase gene was isolated with a combination of standard and inverse PCR techniques. Briefly, Lactobacillus reuteri DNA was cut with restriction enzyme XhoI and ligated. PCR with the primers 7ftf and 8ftfi, using the ligation product as a template, yielded a 290 bp PCR product which was cloned into pCR2.1 and sequenced. This revealed that primer 8ftfi had annealed aspecifically as well as specifically yielding the 290 bp product (see FIG. 5D).

Detail Description Paragraph - DETX:

[0030] At this time, the N-terminal amino acid sequence of a fructosyltransferase enzyme (FTFB) purified from the Lactobacillus reuteri strain 121 was obtained. This sequence consisted of the following 23 amino acids: QVESNNYNGVAEVNTERQANGQI (SEQ ID No. 6). The degenerated primer 19ftf (YNGVAEV) was designed on the basis of a part of this N-terminal peptide sequence and primer 20ftfi was designed on the 290 bp PCR product. PCR with primers 19ftf and 20ftfi gave a 754 bp PCR product (see FIG. 5E), which was

cloned into pCR2.1 and sequenced. Both DNA strands of the entire fructosyltransferase gene were double sequenced. In this way the sequence of a 2.6 kb region of the Lactobacillus reuteri DNA, containing the inul sucrose gene and its surroundings were obtained.

Detail Description Paragraph - DETX:

[0031] The plasmids for expression of the inulosucrase gene in E. coli Top10 were constructed as described hereafter. A 2414 bp fragment, containing the inulosucrase gene starting at the first putative start codon at position 41, was generated by PCR, using primers ftfA1 and ftfA2i. Both primers contained suitable restriction enzyme recognition sites (a NcoI site at the 5'end of ftfA1 and a BglII site at the 3'end of ftfA2i). PCR with Lactobacillus reuteri DNA, Pwo DNA polymerase and primers ftfA1 and ftfA2i yielded the complete inulosucrase gene flanked by NcoI and BglII restriction sites. The PCR product with blunt ends was ligated directly into pCRbluntII-Topo. Using the NcoI and BglII restriction sites, the putative ftfA gene was cloned into the expression vector pBAD, downstream of the inducible arabinose promoter and in frame upstream of the Myc epitope and the His tag. The pBAD vector containing the inulosucrase gene (pSVH101) was transformed to E. coli Top10 and used to study inulosucrase expression. Correct construction of plasmid containing the complete inulosucrase gene was confirmed by restriction enzyme digestion analysis and by sequence analysis, showing an in frame cloning of the inulosucrase gene using the ribosomal binding site provided by the pBAD vector and the first putative start codon (at position 41) of inulosucrase (see FIG. 1).

Detail Description Paragraph - DETX:

[0034] Fructan production by Lactobacillus reuteri was studied with cells grown in MRS-s medium. Product formation was also studied with cell-free extracts of E. coli containing the novel inulosucrase incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37.degree. C.). Fructans were collected by precipitation with ethanol. ¹H-NMR spectroscopy and methylation analysis were performed as described by van Geel-Schutten et al. (1999) Appl. Environ. Microbiol. 65, 3008-3014. The molecular weights of the fructans were determined by high performance size exclusion chromatography coupled on-line with a multi angle laser light scattering and a differential refractive index detector. Fructo-oligosaccharide synthesis was studied in Lactobacillus reuteri culture supernatants and in extracts of E. coli cells containing the novel inulosucrase incubated in reaction buffer (1 mg protein/10 ml buffer, incubated overnight at 37.degree. C.). Glucose and fructose were determined enzymatically as described above and fructo-oligosaccharides produced were analyzed using a Dionex column. The incubation mixtures were centrifuged for 30 min at 10,000.times.g and diluted 1:5 in a 100% DMSO solution prior to injection on a Dionex column. A digest of inulin (DP1-20) was used as a standard. Separation of compounds was achieved with anion-exchange chromatography on a CarboPac Pa1 column (Dionex) coupled to a CarboPac PA1 guard column (Dionex). Using a Dionex GP50 pump the following gradient was generated: % eluent B is 5% (0 min); 35% (10 min); 45% (20 min);

65% (50 min); 100% (54-60 min); 5% (61-65 min). Eluent A was 0.1 M NaOH and eluent B was 0.6 M NaAc in a 0.1 M NaOH solution. Compounds were detected using a Dionex ED40 electrochemical detector with an AU working electrode and a Ag/AgCl reference-electrode with a sensitivity of 300 nC. The pulse program used was: +0.1 Volt (0-0.4 s); +0.7 Volt (0.41-0.60 s); -0.1 Volt (0.61-1.00 s). Data were integrated using a Perkin Elmer Turbochrom data integration system. A different separation of compounds was done on a cation exchange column in the calcium form (Benson BCX4). As mobile phase Ca-EDTA in water (100 ppm) was used. The elution speed was 0.4 ml/min at a column temperature of 85.degree. C. Detection of compounds was done by a refractive index (Jasco 830-RI) at 40.degree. C. Quantification of compounds was achieved by using the software program Turbochrom (Perkin Elmer).

Detail Description Paragraph - DETX:

[0042] A levansucrase enzyme was purified from LB121 cultures grown on media containing maltose using ammonium sulfate precipitation and several chromatography column steps (table 2). Maltose (glucose-glucose) was chosen because both glucansucrase and levansucrase can not use maltose as substrate. LB121 will grow on media containing maltose but will not produce polysaccharide. From earlier experiments it was clear that even with harsh methods the levansucrase enzyme could not be separated from its product levan. These harsh methods included boiling the levan in a SDS solution and treating the levan with HCl and TFA. No levanase enzyme was commercially available for the enzymatic breakdown of levan. Only a single levansucrase was detected in maltose culture supernatants. In order to prove that the enzyme purified from maltose culture supernatant is the same enzyme which is responsible for the levan production during growth on raffinose, biochemical and biophysical tests were performed.

Detail Description Paragraph - DETX:

[0059] FIG. 1: SEQ ID No. 1; The deduced amino acid sequence of the novel inulosucrase of Lactobacillus reuteri (amino acid 1-789). Furthermore, the designations and orientation (<for 3' to 5' and >for 5' to 3') of the primers and the restriction enzymes used for (inverse) PCR, are shown at the right hand side. Putative start codons (ATG, at positions 41 and 68) and stop codon (TAA, at position 2435) are shown in bold. The positions of the primers used for PCR are shown in bold/underlined. The NheI restriction sites (at positions 1154 and 2592) used for inverse PCR are underlined. The primers used and their exact positions in the inulosucrase sequence are shown in table 1. Starting at amino acid 690, the 20 PXX repeats are underlined. At amino acid 755 the LPXTG motif is underlined.

Detail Description Paragraph - DETX:

[0061] FIG. 3: SEQ ID No. 2; The N-terminal and three internal amino acid sequences of the novel levansucrase of Lactobacillus reuteri.

Detail Description Paragraph - DETX:

[0063] FIG. 5: The strategy used for the isolation of the inulosucrase gene from Lactobacillus reuteri 121 chromosomal DNA.

Detail Description Table CWU - DETL:

2TABLE 2 Purification of the Lactobacillus reuteri LB 121 levansucrase (FTFB)
enzyme Protein Total Specific Purification Yield Step (mg) Activity (U)
Activity (U/mg) (fold) (%) Supernatant 128 64 0.5 1 100 Ammonium sulfate
35.2 42 1.2 2.4 65.6 precipitation (65%) Hydroxyl apatite 1.5 30.6 20.4 40.8
47.8 Phenyl superose 0.27 23 85 170 36 Gel Filtration 0.055 10 182 360 16
MonoQ 0.0255 4 176 352 6

Claims Text - CLTX:

9. A process of producing a fructosyltransferase, comprising culturing a host cell according to claim 8 or a Lactobacillus strain containing one or both fructosyltransferases according to claim 1 in a culture medium, and recovering the protein from the culture medium or the cell free extract.

PGPUB-DOCUMENT-NUMBER: 20020122844

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020122844 A1

TITLE: Method of preparing a food product

PUBLICATION-DATE: September 5, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|----------------------|-------------|-------|---------|---------|
| Cooke, David | Bedford | | GB | |
| Foster, Timothy John | Bedford | | GB | |
| Galema, Saskia | Bedford | | GB | |
| Alexandra | Vlaardingen | | NL | |
| Ledeboer, Adrianus | Vlaardingen | | NL | |
| Marinus | | | | |
| Sanders, Jan Willem | | | | |

APPL-NO: 09/ 929569

DATE FILED: August 14, 2001

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|-----------|------------------|-----------------|
| GB | 0020002.2 | 2000GB-0020002.2 | August 14, 2000 |

US-CL-CURRENT: 426/34

ABSTRACT:

A method for preparing a milk-based composition comprising a homopolysaccharide is provided whereby a mixture comprising milk and fermentable sugar is fermented with a homopolysaccharide-producing microorganism under anaerobic conditions, without regulating the pH, and the fermentation is stopped before the pH of the mixture drops below pH 5.5. Food products comprising such compositions are also provided.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0003] Dextrans are homopolysaccharides comprising glucose residues which are produced by the action of the enzyme dextranucrase on sucrose, these enzymes being produced primarily extracellularly by various lactic acid bacteria including strains from the genera Streptococcus, Leuconostoc and Lactobacillus.

Summary of Invention Paragraph - BSTX:

[0010] U.S. Pat. No. 6,004,800 (mentioned above) describes the preparation of a yoghurt of acceptable texture and taste by fermentation of dextran producing *Leuconostoc mesenteroides* ssp. *cremoris* CNCM I-1692 (together with non-thickening strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) in the presence of a milk-sucrose medium until the pH reaches a value of 4.5. This reference also discloses yoghurt formation using the enzyme dextranucrase, produced by *Leuconostoc mesenteroides* ssp *cremoris*, in the presence of sucrose to produce dextran in situ. In another example U.S. Pat. No. 5,308,628 describes the preparation of a thickener-free, yoghurt-based dairy dessert by culturing *Lactobacillus acidophilus*, *Lactobacillus bifidus* and/or *Streptococcus thermophilus* with various milk constituents to a pH between pH 4.3 and pH 5.5, so that the viscosity of the product is increased to an acceptable level.

PGPUB-DOCUMENT-NUMBER: 20020065403

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020065403 A1

TITLE: NEW NUCLEOTIDE SEQUENCES WHICH CODE FOR PCK GENE

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|-----------|-------|---------|---------|
| EIKMANN, BERNHARD | ULM | | DE | |
| RIEDEL, CHRISTIAN | NEU-ULM | | DE | |
| SAHM, HERMANN | JULICH | | DE | |
| MOCKEL, BETTINA | BIELEFELD | | DE | |

APPL-NO: 09/ 455777

DATE FILED: December 7, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|--------------|---------------------|------------------|
| DE | 199 50 409.1 | 1999DE-199 50 409.1 | October 20, 1999 |

US-CL-CURRENT: 536/23.1,435/199 ,435/252.3 ,435/254.11 ,435/320.1 ,435/325 ,435/6 ,435/91.1 ,536/23.2

ABSTRACT:

Isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence chosen from the group consisting of

- polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
- polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for the polypeptide which is expressed by the pck gene contained on vector pKI9mobsacB.DELTA.pck in the deposited E. coli strain DSM 13047,
- polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2,
- polynucleotide which is complementary to the polynucleotides of a), b) or c)

and

e) polynucleotide comprising at least 15 successive bases of the polynucleotide sequence of a), b), c) or d).

----- KWIC -----

Detail Description Paragraph - DETX:

[0104] *E. coli* S17-1 was then transformed with the integration plasmid pK19mobsacB.DELTA.pck (Simon et al., Bio/Technology 1,784-791 (1983)). This strain allows transfer of a plasmid to *Corynebacterium glutamicum* by conjugation (Schfer et al., Journal of Bacteriology 172 (1990) 1663-1666). The lysine production strain *C. glutamicum* MH20-22B was used as the recipient of the conjugation (Schrumpf et al., Applied Microbiology and Biotechnology 37 (1992) 566-571)). Several transconjugants were obtained from the conjugation between *E. coli* S17-1/pK19mobsacB.DELTA.pck and *C. glutamicum* MH20-22B and subsequent selection on Luria-Bertani agar plates with kanamycin (25 $\mu\text{g/ml}$) and nalidixic acid (50 $\mu\text{g/ml}$). For selection for the second recombination event, which is to lead to excision of the vector together with the pck gene, these transconjugants were cultured on antibiotic-free Luria-Bertani complex medium [Sambrook et al; Molecular Cloning, A laboratory manual (1989) Cold Spring Harbour Laboratory Press] with 1% glucose and then plated out on the same medium plus 10% sucrose. The sacB gene present on the vector pK19mobsacB codes for the enzyme **levan sucrase and leads to synthesis of levan** from sucrose. Since levan is toxic to *C. glutamicum*, only *C. glutamicum* cells which have lost the integration plasmid can grow on sucrose-containing medium (Jger et al., Journal of Bacteriology 174 (1992) 5462-5466). 30 sucrose-resistant clones were investigated for their kanamycin sensitivity. For 11 of the clones tested, in addition to the sucrose resistance, the desired kanamycin sensitivity could also be confirmed. In these 11 clones, the vector background had therefore been excised again. Whether the desired deletion had also taken place was tested by analysis by means of the polymerase chain reaction (PCR). For this, chromosomal DNA was liberated from a colony of the starting strain and from colonies of the 11 kanamycin-sensitive clones. For this, the particular colony was removed from the agar plate with a toothpick, suspended in 50 μl H₂O and incubated for 5 minutes at 95 $^{\circ}\text{C}$. 1 μl portions of the resulting solution were each employed as templates in the PCR.

PGPUB-DOCUMENT-NUMBER: 20020064850

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020064850 A1

TITLE: Nucleic acid molecules encoding enzymes having fructosyltransferase activity, and their use

PUBLICATION-DATE: May 30, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|--------|-------|---------|---------|
| Heyer, Arnd G. | Berlin | | DE | |
| Rehm, Jochen | Berlin | | DE | |
| Wendenburg, Regina | Berlin | | DE | |

APPL-NO: 09/ 798791

DATE FILED: March 2, 2001

RELATED-US-APPL-DATA:

child 09798791 A1 20010302 parent continuation-of PCT/EP99/06319 19990827 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|--------------|---------------------|-------------------|
| DE | DE19840028.4 | 1998DE-DE19840028.4 | September 2, 1998 |

US-CL-CURRENT: 435/193,435/254.3 ,435/320.1 ,435/325 ,536/23.2

ABSTRACT:

Nucleic acid molecules are described which encode polypeptides having the enzymatic activity of a fructosyltransferase. Also, vectors, host cells and transgenic plants are described which contain such nucleic acid molecules. Furthermore, processes for producing polyfructose, particularly that of the inulin type, using the hosts described and/or the fructosyltransferase produced by them are described.

----- KWIC -----

Abstract Paragraph - ABTX:

Nucleic acid molecules are described which encode polypeptides having the enzymatic activity of a fructosyltransferase. Also, vectors, host cells and transgenic plants are described which contain such nucleic acid molecules.

Furthermore, processes for **producing polyfructose, particularly that of the inulin** type, using the hosts described and/or the **fructosyltransferase** produced by them are described.

Summary of Invention Paragraph - BSTX:

[0001] The present invention relates to nucleic acid molecules encoding polypeptides having the enzymatic activity of a **fructosyltransferase**. The invention furthermore relates to vectors and hosts containing such nucleic acid molecules. The present invention also relates to processes for **producing fructosyltransferase and the production of polyfructoses of the inulin** type in various host organisms or in vitro, as well as the **fructosyltransferase** encoded by the nucleic acid molecules described which may be used to **produce polyfructoses of the inulin** type.

Summary of Invention Paragraph - BSTX:

[0004] Various processes have been described for **producing fructan** polysaccharides in plants by expressing **fructosyltransferases** of bacterial origin or for producing polyfructoses of medium chain length by expressing **fructosyltransferases** of plant origin. PCT/US89/02729, e.g., describes the possibility of generating carbohydrate polymers, particularly dextrane or polyfructose, in transgenic plants, namely specifically in the fruit of transgenic plants. In order to generate plants that are modified in that way it is proposed to use levan sucrose from microorganisms, particularly from *Aerobacter levanicum*, *Streptococcus salivarius* and *Bacillus subtilis*, or of dextrane sucrases from *Leuconostoc mesenteroides*. Neither the generation of the active enzyme, nor that of **levan or dextrane nor the production** of transgenic plants is described.

Summary of Invention Paragraph - BSTX:

[0005] PCT/EP93/02110 discloses a method for producing transgenic plants which express the *lsc* gene of the levan **sucrase** from the gram-negative bacterium *Erwinia amylovora*. The plants **produce a high-molecular, highly branched levan**.

Summary of Invention Paragraph - BSTX:

[0006] PCT/NL93/00279 describes the transformation of plants with chimeric genes that contain the *sacB* gene from *Bacillus subtilis*. Such plants **produce a branched levan**. The bacterial **fructosyltransferases** used in PCT/US89/02729, PCT/EP93/02110 and PCT/NL93/00279 **synthesize levan**, a .beta.-2,6 linked fructosyl polymer which has numerous .beta.-2,1 branchings. Due to the numerous branchings, however, levan involves decisive disadvantages for the technical processing and is therefore much less in demand as technical starting material than inulin which displays .beta.-2,1 linkings. Presently, only one bacterial gene is known the gene **product of which is involved in the synthesis of inulin**, said gene being the *ftf* gene from *Streptococcus mutans*.

PCT/NL93/00279 describes the transformation of plants with said gene which synthesize high-molecular inulin but in such small amounts that it cannot be economically utilized. PCT/EP97/102195, too, describes a process for producing transgenic, inulin-producing plants with the *ftf* gene from *Streptococcus* mutans. Like with the plants described in PCT/NL93/00279 the yield of high-molecular inulin is low. While it is possible to express the gene in plants if the gene was genetically engineered beforehand, the yield in inulin that can be obtained from transgenic plants is so low that the transgenic plants cannot be economically utilized.

Summary of Invention Paragraph - BSTX:

[0007] Furthermore, PCT/NL96/00012 discloses DNA sequences which encode carbohydrate polymer-synthesizing enzymes as well as the production of transgenic plants using said DNA sequences. The sequences disclosed originate from *Helianthus tuberosus*. In accordance with PCT/NL96/00012 the sequences disclosed can be used to modify the fructan profile of petunia and potato but also of *Helianthus tuberosus* itself. Expression of the sequences disclosed which encode a sucrose-dependent sucrose fructosyltransferase (SST) or a fructan fructosyl transferase in transgenic plants allows the production of inulin. The average polymerization degree of the inulin is, however, DP=6 to DP=10. With such a polymerization degree the inulin cannot be considered long-chain. The process described in PCT/NL96/00012 does not allow to produce high-molecular inulin.

Summary of Invention Paragraph - BSTX:

[0009] DE 197 08 774.4 relates to the production of 1-kestose and nystose using enzymes having fructosyl polymerase activity. The tri- and tetrasaccharide may be produced in transgenic plants. The yield is high and in potato corresponds to the cellular content of sucrose. However, the production of longer-chain inulin is not described. The synthesis of polyfructoses by fungi is also discussed in many publications. Barthomeuf and Pourrat (Biotechnology Letters 17 (1995), 911-916), describe, e.g., an enzyme preparation of *Penicillium rugulosum* which has fructosyltransferase activity. The preparation exhibits various enzymatic properties and therefore does not represent a pure fructosyltransferase. DNA sequences of the fructosyltransferase gene are not known. Cairns et al. (New Phytologist 129 (1995), 299-308) describe a transient synthesis of tri-, tetra- and pentasaccharides from sucrose in the culture medium of *Monographella nivalis*. The underlying enzymatic activity appears to be of mainly hydrolytic nature since the polyfructoses are degraded again by the enzyme with increasing substrate exhaustion. Since no DNA sequence is known it is not possible to assess--relying on the homology with fructofuranosidases (invertases) as reference--whether a fructosyltransferase in the proper sense or an invertase is present.

Summary of Invention Paragraph - BSTX:

[0019] The sequence depicted in Seq ID No.1 encodes a sucrose-dependent fructan

fructosyltransferase from *Aspergillus sydowi* which leads in plant cells to the synthesis of a long-chain polyfructan of the inulin type. It was surprisingly found that it is possible to produce long-chain polyfructans of the inulin type in high yields in host organisms, specifically in transgenic plants, bacteria or fungi when using said sequences.

Summary of Invention Paragraph - BSTX:

[0025] A polypeptide encoded by a nucleic acid molecule according to the invention with the activity of a fructosyltransferase leads to the synthesis of polyfructose and particularly in plant cells to the synthesis of polyfructose of the inulin type (hereinafter also referred to as inulin).

Summary of Invention Paragraph - BSTX:

[0094] The invention furthermore relates to proteins possessing fructosyltransferase activity, i.e., fructosyltransferases encoded by the nucleic acid molecules according to the invention or obtainable by the method according to the invention. The fructosyltransferases according to the invention may preferably be used to produce polyfructoses of the inulin type. They may also serve to produce antibodies which may be used to detect and/or purify fructosyltransferases.

Summary of Invention Paragraph - BSTX:

[0096] Another subject matter of the present invention are processes for producing polyfructoses, particularly of the inulin type, whereby host cells according to the invention, or host organisms containing them, are cultivated under conditions allowing expression of the fructosyltransferase according to the invention as well as synthesis of polyfructose.

Summary of Invention Paragraph - BSTX:

[0097] By the provision of the nucleic acid molecules according to the invention it has become possible to produce--by way of methods of gene technology--polyfructoses, particularly of the inulin type, in organisms, such as has not been possible so far using conventional methods. It is thus possible to express the nucleic acid molecules according to the invention in hosts such as bacteria, fungi or plant cells in order to increase the activity of the corresponding fructosyltransferase or to introduce it into cells that normally do not express said enzyme. Due to the expression or additional expression of at least one nucleic acid molecule according to the invention the host cells according to the invention synthesize polyfructose, particularly of the inulin type. Another subject matter of the present invention are therefore the polyfructoses, particularly of the inulin type, obtainable from the host cells according to the invention as well as obtainable from the propagation material and, for plants, obtainable from the plants and their harvest products.

Summary of Invention Paragraph - BSTX:

[0109] The present invention furthermore relates to a process for **producing polyfructose, particularly of the inulin** type, characterized in that a fungal **fructosyltransferase** is used for producing the polyfructose, or a host organism expressing a fungal **fructosyltransferase**. Preferably, **fructosyltransferases** according to the invention or host cells according to the invention can be used. The present invention shows for the first time that it is possible to use such fungal **fructosyltransferases for producing polyfructose of the inulin** type.

Summary of Invention Paragraph - BSTX:

[0110] Finally, the present invention relates to the use of fungal **fructosyltransferases for producing polyfructose, particularly of the inulin** type.

Detail Description Paragraph - DETX:

[0147] In FIG. 6 one can see that E coli clones that are transformed with a pBluescript vector without insert are not capable of converting sucrose (lane 1), while those transformed with plasmid pas1 synthesize the trisaccharide kestose. Clones that are transformed with plasmid pSK-as1 are also capable of synthesizing higher oligomers (lanes 3-6). Simultaneously, fructose residues are transferred to water, thereby forming fructose. Said conversion is catalyzed by the SFT from *Aspergillus sydowi*. FIG. 7 shows that protein extracts of yeasts that are transformed with plasmid 112-as1 may **synthesize fructan**. The **fructosyltransferase** activity is higher in these yeasts than in those which were transformed with the construct 112-as1L. The latter--due to the smaller **fructosyltransferase** activity achieved within the time available in the experiment--can only synthesize the trisaccharide. The size of the fructans synthesized depends on the reaction time and the **fructosyltransferase** activity. FIG. 8 demonstrates that the size of the **fructan synthesized** in the extracts of transformed tobacco protoplasts depends at the given reaction time on the amount of **fructosyltransferase** activity achieved, which in turn depends on the amount plasmid pA7-as1 transformed. In lanes 3-5 one can see that oligo- and polymers with a DP>7 have been synthesized which upon chromatography do not migrate from the site of application. The same holds true for plant extracts from stably transformed plants as shown in FIGS. 9 and 10.

Claims Text - CLTX:

21. A process for **producing polyfructose, particularly that of the inulin** type comprising: (a) cultivating a host cell according to claim 8 or 9 or a host containing such a cell under conditions allowing the production of **fructosyltransferase** and converting sucrose optionally added or a substrate

equivalent to polyfructose; and (b) obtaining the polyfructose produced in that manner from the cultivated cells, the host or from the medium.

Claims Text - CLTX:

22. A process for **producing polyfructose, particularly that of the inulin** type, comprising: (a) contacting sucrose or an equivalent substrate with a **fructosyltransferase** according to claim 19 under conditions allowing the conversion to polyfructose; and (b) obtaining the polyfructose produced in that manner.

Claims Text - CLTX:

26. Use of the **fructosyltransferase** according to claim 19 for **producing polyfructose, particularly that of the inulin** type.

PGPUB-DOCUMENT-NUMBER: 20020061579

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020061579 A1

TITLE: Counter selection strategy for Gram-negative bacteria

PUBLICATION-DATE: May 23, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|---------------------|---------|-------|---------|---------|
| Farrand, Stephen K. | Seymour | IL | US | |
| Staswick, Paul E. | Lincoln | NE | US | |
| Clemente, Thomas E. | Lincoln | NE | US | |

APPL-NO: 09/ 924101

DATE FILED: August 7, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60223920 20000809 US

US-CL-CURRENT: 435/252.2,536/23.2 ,800/294

ABSTRACT:

A Gram-negative bacterium useful for genetically engineering plants is provided. The Gram-negative bacterium contains, as part of genome, an inducible regulatory sequence operatively linked to a nucleotide sequence encoding a levansucrase. Alternatively, the Gram-negative bacterium comprises a recombinant nucleic acid construct containing an inducible regulatory sequence operatively linked to a nucleotide sequence encoding a levansucrase. Also provided are recombinant nucleic acid constructs comprising an inducible regulatory sequence operatively coupled to a nucleotide sequence encoding a levansucrase and a method for transforming plants using the Gram-negative bacterium of the present invention.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0004] The *saeB* gene from *Bacillus subtilis* encodes for the enzyme levansucrase, which hydrolyzes sucrose to produce the polysaccharide levan, the presence of which causes the lysis of several Gram-negative bacteria, and in particular *Agrobacterium* (Gay et al. (1985)) *J. Bacteriol.* 164:918-921). Expression of *sacB* is controlled by its regulatory sequence *sacR*. The present

inventors have discovered that the introduction of a sequence encoding a levansucrase and in particular the sacB open reading frame (ORF), under strict control of an inducible regulatory sequence allows for the selection of Agrobacterium cells without the use of antibiotics. Thus, the present invention overcomes the problems of cost and negative effects on plant culture associated with the use of antibiotics for counter selection of the Agrobacterium cells.

Summary of Invention Paragraph - BSTX:

[0022] The present invention provides a method for transforming plant cells and constructs and bacteria useful in said method. The invention involves the insertion of a sequence encoding a levansucrase, and in particular the sacB gene ORF, under strict control of an inducible regulatory sequence into a Gram-negative bacterium and in particular Agrobacterium tumefaciens. The sacB gene encodes levansucrase (sucrose 2,6-.beta.-D-fructan 6-.beta.-D-glucosyltransferase; (IC 2.3.1.10)), a 50 kD enzyme secreted by B. subtilis after induction by sucrose. Levansucrase catalyzes transfructorylation from sucrose to various acceptors. The two main physiological reactions resulting are, 1) levan synthesis and 2) sucrose hydrolysis. Since sucrose is the primary carbon source used in most plant tissue culture medium formulations, the present invention allows for the efficient counter selection of the bacterium without the use of antibiotic supplements.

Summary of Invention Paragraph - BSTX:

[0030] Another aspect provides method for transforming a plant cell using an Agrobacterium tumefaciens bacterium of the present invention as a vector. In general the method involves obtaining an A. tumefaciens strain whose genome includes a nucleotide sequence encoding a levansucrase operatively linked to an inducible regulatory sequence as described above or an A. tumefaciens strain that contains a recombinant nucleotide sequence encoding a levansucrase operatively linked to an inducible regulatory sequence as described above. The nucleotide sequence(s) of interest that are to be transferred to the plant cell can be inserted within the T-DNA element and introduced either directly to the resident Ti plasmid or separately using a binary plasmid strategy. Methods for the introduction of exogenous nucleotide sequences into the T-DNA element and the use of the derived Agrobacterium transconjugant to transform plant cells are well known in the art (see, Maliga et al. Methods in Plant Molecular Biology, Cold Spring Harbor Laboratory Press, 1995). Once the sequence to be delivered to the plant cell is assembled into the T-DNA element and introduced either directly to the resident Ti plasmid or via the binary vector strategy to the Agrobacterium of the present invention, the bacterium is subsequently used to inoculate plant cells either by direct injection or by co-cultivating the bacterium of the present invention with individual plant cells or pieces of plants such as leaf discs. Co-cultivation is carried out in medium supplemented with a carbon source, preferably glucose, for a sufficient amount of time to allow the T-DNA element to be mobilized from the bacterium to the plant cell genome. The co-cultivation period is determined empirically, but generally ranges from one to seven days. Co-cultivation periods may vary for a

particular plant species, but determinations are routine in the art and can be made by one of ordinary skill in the art without undue experimentation. Following co-cultivation, the transforming bacteria are counter selected prior to the regeneration of the plant cells to whole plants. Typically, the transforming bacteria are removed by using antibiotic supplements to the regeneration medium. In the present invention, however, an inducing agent that activates the promoter linked to the levansucrase coding region is added to sucrose amended regeneration medium. Activation of the inducible promoter results in the **production of levan** which causes the lysis of the Agrobacterium cells, thus providing efficient counter selection strategy. As a result, the regeneration of whole plants from the inoculated plant cells can be carried out, in the absence of antibiotics typically used to counter select Agrobacterium cells, following standard protocols (see, Maliga et al. supra).

PGPUB-DOCUMENT-NUMBER: 20020037260

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020037260 A1

TITLE: Compositions for treating biofilm

PUBLICATION-DATE: March 28, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|------------------|-------|---------|---------|
| Budny, John A. | Westlake Village | CA | US | |
| Budny, Matthew J. | Westlake Village | CA | US | |

APPL-NO: 09/ 876248

DATE FILED: June 6, 2001

RELATED-US-APPL-DATA:

child 09876248 A1 20010606 parent continuation-in-part-of 09587818 20000606 US
PENDING child 09587818 20000606 US parent continuation-in-part-of 09249674
19990212 US GRANTED parent-patent 6159447 US child 09249674 19990212 US parent
continuation-in-part-of 08951393 19971016 US GRANTED parent-patent 5871714 US

US-CL-CURRENT: 424/49,424/50 ,424/94.63 ,514/253.08 ,514/8

ABSTRACT:

A composition for treating a biofilm comprises a first anchor enzyme component to degrade biofilm structures and a second anchor enzyme component having the capability to act directly upon the bacteria for a bactericidal effect.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation-in-part of U.S. application Ser. No. 09/587,818 filed Jun. 06, 2000, which is a continuation-in-part of U.S. application Ser. No. 09/249,674 filed Feb. 12, 1999 (issued as U.S. Pat. No. 6,159,447 on Dec. 12, 2000), which is a continuation-in-part of U.S. application Ser. No. 08/951,393 filed Oct. 16, 1997 (issued as U.S. Pat. No. 5,871,714 on Feb. 16, 1999), both of which are incorporated herein by reference.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0039] **Dextranucrase**; Starch-synthesizing enzymes; Cellulose-synthesizing enzymes; Chitin-synthesizing enzymes; Glycogen-synthesizing enzymes; Pectate synthetase; Glycosyl transferase-binding domains (glucan-, mutan-, **levan-**, **Polygalact syl-synthesizing** enzymes; et al.

Claims Text - CLTX:

17. A composition as claimed in claim 7 wherein the anchor is selected from the group consisting of: concanavalin A, wheat germ agglutinin, other lectins, elastase, amylose binding protein, binding domains from enzymes, **dextranucrase**, starch-synthesizing enzymes, cellulose-synthesizing enzymes, chitin-synthesizing enzymes, glycogen-synthesizing enzymes, pectate synthetase, glycosyl transferase-binding domains (glucan-, mutan-, **levan-**, **polygalactosyl-synthesizing** enzymes).

PGPUB-DOCUMENT-NUMBER: 20020023279

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020023279 A1

TITLE: Forages

PUBLICATION-DATE: February 21, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|---------------|-------|---------|---------|
| Loiselle, Francois J. | Clive | IA | US | |
| Nichols, Scott E. | Johnston | IA | US | |
| Jenkins, Colin Leslie | Evatt | | AU | |
| Dow | Murrumbateman | | AU | |
| Simpson, Richard J. | | | | |

APPL-NO: 09/ 844408

DATE FILED: April 27, 2001

RELATED-US-APPL-DATA:

child 09844408 A1 20010427 parent continuation-of 09653884 20000901 US
ABANDONED child 09653884 20000901 US parent continuation-of 09350649 19990709
US ABANDONED child 09350649 19990709 US parent continuation-in-part-of 08979514
19971126 US GRANTED parent-patent 5985666 US child 08979514 19971126 US parent
continuation-in-part-of 08478704 19950607 US ABANDONED child 08979514 19971126
US parent continuation-in-part-of 08485243 19950607 US GRANTED parent-patent
5712107 US child 08979514 19971126 US parent continuation-in-part-of 08482711
19950607 US ABANDONED

US-CL-CURRENT: 800/284,800/320

ABSTRACT:

A transgenic plant cell is provided containing a DNA molecule encoding an enzyme selected from the group consisting of fructosyltransferase, glucosyltransferase B, mutants of glucosyltransferase B, glucosyltransferase C, glucosyltransferase D, mutants of glucosyltransferase D and functional fragments of each enzyme. A transgenic plant regenerated from the plant cell is also provided. A method of improving the ensilability and the nutritional value of plants is also provided comprising introducing into the cells of the plant an expression cassette comprising the above DNA molecule operably linked to a promoter functional in the cells of the plant to yield transformed plant cells, and regenerating a transformed plant from the transformed cells. The transformed plants also provide improved digestibility in ruminants.

[0001] This application is a continuation of co-pending application U.S. Ser.

No. 09/350,649 filed Jul. 7, 1999 which is a continuation of U.S. Ser. No. 08/979,514 filed Nov. 26, 1997. Which application is a continuation-in-part of co-pending applications U.S. Ser. Nos. 08/478,704; 08,485,243; 08/482,711 all filed Jun. 7, 1995.

----- KWIC -----

Detail Description Paragraph - DETX:

[0065] Sprenger, N., et al., Purification, cloning, and functional expression of sucrose:fructan 6-fructosyltransferase, a key enzyme of fructan synthesis in barley, Proc. Natl. Acad. Sci. 92(25):11652-11656 (1995).

Detail Description Paragraph - DETX:

[0071] The production of the present transgenic plants is performed according to methods of transformation that are well known in the art. The glucans and fructans are synthesized by insertion of an expression cassette containing a structural gene which, when transcribed and translated, yields a glucosyltransferase or fructosyltransferase enzyme that produces the desired glucan or fructan.

PGPUB-DOCUMENT-NUMBER: 20020019997

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020019997 A1

TITLE: Forages

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-----------------------|---------------|-------|---------|---------|
| Loiselle, Francois J. | Clive | IA | US | |
| Nichols, Scott E. | Johnson | IA | US | |
| Jenkins, Colin Leslie | Evatt | | AU | |
| Dow | Murrumbateman | | AU | |
| Simpson, Richard J. | | | | |

APPL-NO: 09/ 879486

DATE FILED: June 12, 2001

RELATED-US-APPL-DATA:

child 09879486 A1 20010612 parent continuation-of 09653885 20000901 US
ABANDONED child 09653885 20000901 US parent continuation-of 09350649 19990709
US ABANDONED child 09350649 19990709 US parent continuation-of 08979514
19971126 US GRANTED parent-patent 5985666 US child 08979514 19971126 US parent
continuation-in-part-of 08478704 19950607 US ABANDONED child 08979514 19971126
US parent continuation-in-part-of 08485243 19950607 US GRANTED parent-patent
5712107 US child 08979514 19971126 US parent continuation-in-part-of 08482711
19950607 US ABANDONED

US-CL-CURRENT: 800/278,800/320 ,800/320.2 ,800/320.3

ABSTRACT:

A transgenic plant cell is provided containing a DNA molecule encoding an enzyme selected from the group consisting of fructosyltransferase, glucosyltransferase B, mutants of glucosyltransferase B, glucosyltransferase C, glucosyltransferase D, mutants of glucosyltransferase D and functional fragments of each enzyme. A transgenic plant regenerated from the plant cell is also provided. A method of improving the ensilability and the nutritional value of plants is also provided comprising introducing into the cells of the plant an expression cassette comprising the above DNA molecule operably linked to a promoter functional in the cells of the plant to yield transformed plant cells, and regenerating a transformed plant from the transformed cells. The transformed plants also provide improved digestibility in ruminants.

[0001] This application is a continuation of co-pending application U.S. Ser.

No. 09/350,649 filed Jul. 7, 1999 which is a continuation of U.S. Ser. No 08/979,514 filed Nov. 26, 1997. Which application is a continuation-in-part of co-pending applications U.S. Ser. Nos. 08/478,704; 08,485,243; 08/482,711 all filed Jun. 7, 1995.

----- KWIC -----

Detail Description Paragraph - DETX:

[0067] Sprenger, N., et al., Purification, cloning, and functional expression of sucrose:fructan 6-fructosyltransferase, a key enzyme of fructan synthesis in barley, Proc. Natl. Acad. Sci. 92 (25), 11652-11656 (1995).

Detail Description Paragraph - DETX:

[0073] The production of the present transgenic plants is performed according to methods of transformation that are well known in the art. The glucans and fructans are synthesized by insertion of an expression cassette containing a structural gene which, when transcribed and translated, yields a glucosyltransferase or fructosyltransferase enzyme that produces the desired glucan or fructan.

PGPUB-DOCUMENT-NUMBER: 20010016572

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20010016572 A1

TITLE: METHOD FOR PREPARING A POLYDISPERSED SACCHARIDE COMPOSITION AND
RESULTING POLYDISPERSED SACCHARIDE COMPOSITION

PUBLICATION-DATE: August 23, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|-------------------|-----------|-------|---------|---------|
| DE LEENHEER, LEEN | TERVUREN | | BE | |
| BOOTEN, KARL | GEETBEETS | | BE | |

APPL-NO: 09/ 230769

DATE FILED: February 1, 1999

CONTINUED PROSECUTION APPLICATION: CPA

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|----------|-----------------|----------------|
| BE | 09600676 | 1996BE-09600676 | August 1, 1996 |

PCT-DATA:

APPL-NO: PCT/BE97/00087

DATE-FILED: Jul 25, 1997

PUB-NO:

PUB-DATE:

371-DATE:

102(E)-DATE:

US-CL-CURRENT: 514/23,514/25 ,514/53 ,514/54 ,536/123 ,536/128

ABSTRACT:

The invention concerns a method for preparing a polydispersed saccharide composition poor in glucose (G), fructose (F) and saccharose (GF) containing at least 93.5% by weight per dry matter of fructo-oligosaccharides constituted by a chain of fructose units with a terminal glucose of formula GF.sub.n, n and m being between 2 and 20 and comprising a content of glucose, fructose and saccharose in total less than 5% by weight per dry matter. The method consists in subjecting a substance containing fructanes to partial hydrolysis, the said substance containing fructanes of an average degree of polymerisation not less than 7 and containing glucose, fructose and saccharose in total at most 3.5% by weight per dry matter.

----- KWIC -----

Summary of Invention Paragraph - BSTX:

[0027] The DP of an **inulin produced** by microorganisms may vary up to values of the order of 60,000. Such an **inulin is, for example, synthesized** from saccharose by *Aspergillus sydowi* conidia in the presence of L-cysteine, as described in the article "Characteristics and Applications of a Polyfructan Synthesized from Sucrose by *Aspergillus sydowi* conidia" (T. Harada et al., Food Hydrocolloids, Vol. 7, No. 1, pp. 23-28 (1993)). The **production of a "bacterial" inulin by a fructosyltransferase** from *Streptococcus mutans* is described in "Genetic and Antigenic Comparison of *Streptococcus mutans* **Fructosyltransferase** and Glucan-binding Protein" (J. Aduse-Opoku, FEMS Microbiology Letters 59, pp. 279-282 (1989)).

Summary of Invention Paragraph - BSTX:

[0031] In the case where the fructans are inulin, an enzymatic preparation having an endo-inulinase activity is used. Such preparations are known and can be obtained i.a. from cultures of *Penicillium*, *Aspergillus*, *Fusarium* or *Chrysosporium* (see also the document "The **production of Fructooligosaccharides from Inulin** or Sucrose Using Inulinase or **Fructosyltransferase** from *Aspergillus ficuum*" (Denpun Kagaku, Vol. 36, No. 2, pp. 103-111 (1989)), incorporated herein by reference).

US-PAT-NO: 6423833

DOCUMENT-IDENTIFIER: US 6423833 B1

TITLE: Functional sugar polymers from inexpensive sugar sources and apparatus for preparing same

DATE-ISSUED: July 23, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|--------|-------|-----------|---------|
| Catani; Steven J. | Athens | GA | 30606 | N/A |
| Laurenzo; Kathleen S. | Athens | GA | 30605 | N/A |
| Navia; Juan L. | Athens | GA | 30606 | N/A |
| Walkup; Robert E. | Novi | MI | 48374-364 | N/A |

8

APPL-NO: 09/ 305788

DATE FILED: May 4, 1999

PARENT-CASE:

This application claims benefit of provisional application 60/084,281 filed May 5, 1998 and is a continuation-in-part of U.S. Pat. No. 5,968,365, filed Jan. 15, 1997.

US-CL-CURRENT: 536/18.5; 435/101 ; 435/97 ; 536/123 ; 536/123.1 ; 536/124

ABSTRACT:

A process for preparing functional sugar polymers comprising transferring a monosaccharide or oligosaccharide to an acceptor, removing by-products, separating polymers which have not achieved the desired chain length and recycling these underdeveloped polymers, and an apparatus for producing same.

17 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 6

----- KWIC -----

Detailed Description Text - DETX:

The system is also very flexible in how enzyme can be utilized. In one form, fresh enzyme is added as an ingredient and deactivated enzyme periodically

removed using a membrane to recover any sugars carried with the purge. In other cases the organism which produces the enzyme can be grown in the reactor. This is the case when the organism can grow at the reaction conditions and when it naturally over-expresses and excretes the required enzyme. In the case of inulin, yeasts that produce the fructose polymerization enzyme, e.g.; *Aspergillus niger*, *A. japonicus* *Pullularia* or *Aureobasidium pullulans*, *Saccharomyces cerevisiae*, etc., behave as just described. Further, they grow on the by-product of the reaction path, glucose. Processes which use fructosyl-transferases derived from *Pullularia* or *Aureobasidium pullulans*, and certain *Aspergillus* strains, useful in the preparation of fructans, are disclosed in the following: U.S. Pat. No. 4,309,505; U.S. Pat, No. 4,317,880; U.S. Pat, No. 4,335,207; U.S. Pat. Nos. 4,356,262; 4,423,150; U.S. Pat. No. 4,849,356.

US-PAT-NO: 6420151

DOCUMENT-IDENTIFIER: US 6420151 B1

TITLE: Nucleotide sequences which code for the pck gene

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-----------|-------|----------|---------|
| Eikmanns; Bernard | Ulm | N/A | N/A | DE |
| Riedel; Christian | Neu-Ulm | N/A | N/A | DE |
| Sahm; Hermann | Julich | N/A | N/A | DE |
| Mockel; Bettina | Bielefeld | N/A | N/A | DE |

APPL-NO: 09/ 455777

DATE FILED: December 7, 1999

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|------------|------------------|
| DE | 199 50 409 | October 20, 1999 |

US-CL-CURRENT: 435/194; 435/183 ; 435/252.3 ; 435/320.1 ; 435/69.1 ; 536/23.2

ABSTRACT:

Isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence chosen from the group consisting of a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2, b) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for the polypeptide which is expressed by the pck gene contained on vector pK19mobsacB.DELTA.pck in the deposited E.coli strain DSM 13047, c) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least 70% to the amino acid sequence of SEQ ID No. 2, d) polynucleotide which is complementary to the polynucleotides of a), b) or c) and e) polynucleotide comprising at least 15 successive bases of the polynucleotide sequence of a), b), c) or d).

8 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Detailed Description Text - DETX:

E. coli S17-1 was then transformed with the integration plasmid pK19mobsacB.DELTA.pck (Simon et al., *Bio/Technology* 1,784-791 (1983)). This strain allows transfer of a plasmid to *Corynebacterium glutamicum* by conjugation (Schafer et al., *Journal of Bacteriology* 172 (1990) 1663-1666). The lysine production strain *C. glutamicum* MH20-22B was used as the recipient of the conjugation (Schrumpf et al., *Applied Microbiology and Biotechnology* 37 (1992) 566-571)). Several transconjugants were obtained from the conjugation between *E. coli* S17-1/pK19mobsacB.DELTA.pck and *C. glutamicum* MH20-22B and subsequent selection on Luria-Bertani agar plates with kanamycin (25 $\mu\text{g/ml}$) and nalidixic acid (50 $\mu\text{g/ml}$). For selection for the second recombination event, which is to lead to excision of the vector together with the pck gene, these transconjugants were cultured on antibiotic-free Luria-Bertani complex medium [Sambrook et al; *Molecular Cloning, A laboratory manual* (1989) Cold Spring Harbour Laboratory Press] with 1% glucose and then plated out on the same medium plus 10% sucrose. The sacB gene present on the vector pK19mobsacB codes for the enzyme levan sucrase and leads to synthesis of levan from sucrose. Since levan is toxic to *C. glutamicum*, only *C. glutamicum* cells which have lost the integration plasmid can grow on sucrose-containing medium (Jager et al., *Journal of Bacteriology* 174 (1992) 5462-5466). 30 sucrose-resistant clones were investigated for their kanamycin sensitivity. For 11 of the clones tested, in addition to the sucrose resistance, the desired kanamycin sensitivity could also be confirmed. In these 11 clones, the vector background had therefore been excised again. Whether the desired deletion had also taken place was tested by analysis by means of the polymerase chain reaction (PCR). For this, chromosomal DNA was liberated from a colony of the starting strain and from colonies of the 11 kanamycin-sensitive clones. For this, the particular colony was removed from the agar plate with a toothpick, suspended in 50 μl H₂O and incubated for 5 minutes at 95 $^{\circ}\text{C}$. 1 μl portions of the resulting solution were each employed as templates in the PCR. Oligonucleotides which cover the regions from nucleotide 2136 to 2158 and from 3815 to 3793 in SEQ ID No. 1 were used as primers. The PCR conditions were: prior denaturing: 150 seconds at 94 $^{\circ}\text{C}$; denaturing 60 seconds at 94 $^{\circ}\text{C}$; hybridization 30 seconds at 60 $^{\circ}\text{C}$; amplification 120 seconds at 72 $^{\circ}\text{C}$; 30 cycles, end extension 240 seconds at 72 $^{\circ}\text{C}$. C. On the basis of the primers chosen, a PCR product of 1.68 kb was expected in the batch with the DNA of the starting strain. A PCR product of 0.61 kb was expected in the PCR with the pck deletion mutant. A PCR product 0.61 kb in size was obtained with one clone. The desired deletion of the internal 1071 bp pck fragment in this clone was thereby demonstrated. The clone was called MH20-22B.DELTA.pck. The 1.68 kb PCR product was detected in the batches of the other clones. In these, the vector had thus been excised such that the genomic starting situation was re-established.

US-PAT-NO: 6399119

DOCUMENT-IDENTIFIER: US 6399119 B1

TITLE: Process for obtaining improved structure build-up of baked products

DATE-ISSUED: June 4, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------------|------------------|-------|----------|---------|
| Vandamme; Erik Jerome | Gent | N/A | N/A | BE |
| Renard; Christian Emile | Waver | N/A | N/A | BE |
| Florius G. | Roosdaal | N/A | N/A | BE |
| Arnaut; Filip Remi | Herselt-Blauberg | N/A | N/A | BE |
| Jules | Fleron | N/A | N/A | BE |
| Vekemans; Nicole | | | | |
| Melanie Francine | | | | |
| Tossut; Pierre Patrick | | | | |
| Aldo | | | | |

APPL-NO: 09/ 273404

DATE FILED: March 22, 1999

PARENT-CASE:

This application is a continuation of application Ser. No. 08/802,196 filed Feb. 14, 1997, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-------------------|
| BE | 09600136 | February 15, 1994 |

US-CL-CURRENT: 426/18; 426/20 ; 426/496 ; 426/546 ; 426/94

ABSTRACT:

A process for obtaining improved structure build-up of baked products includes the steps of incorporating a sufficient amount of exopolysaccharides into a dough to show a rise in viscosity with time and thereafter maintaining the achieved viscosity. New dextrans and new micro-organisms producing them can be used in the process. Thus, the dough and baked products containing these dextrans are produced.

10 Claims, 7 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Claims Text - CLTX:

10. A process according to claim 4, wherein the dextrans are synthesized with the help of a dextran sucrase produced by the **lactic acid bacteria**.

US-PAT-NO: 6365800

DOCUMENT-IDENTIFIER: US 6365800 B1

TITLE: Transgenic crops accumulating fructose polymers and methods for their production

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------|----------------|-------|----------|---------|
| Caimi; Perry G. | Kennett Square | PA | N/A | N/A |

APPL-NO: 09/ 245323

DATE FILED: February 5, 1999

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/077,727, filed Mar. 12, 1998.

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/419 ; 536/23.6 ; 800/287 ; 800/320 ; 800/320.1

ABSTRACT:

A method for producing fructose polymers of various lengths through expression of plant-derived FTF genes in transgenic monocot plants is disclosed. Also disclosed are transgenic monocot plants seeds derived from said plants wherein the level of fructan that accumulates in the cells of the transgenic monocot plants and seeds is increased when compared to the level of fructan that accumulates in the cells of monocot plants and that do not contain the instant chimeric gene(s) encoding plant-derived FTF genes.

19 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Brief Summary Text - BSTX:

Transgenic crops accumulating fructan through expression of chimeric fructosyltransferase (FTF) genes would have a significant advantage over native fructan-storing plants by making use of established breeding programs, pest

resistance and adaptation to a variety of growing regions throughout the world. Examples of **fructan synthesis** in transgenic plants containing genes from bacterial species, such as *Bacillus*, *Streptococcus* and *Erwinia* have been reported (Caimi et al., (1996) *Plant Physiol.* 110:355-363; Ebskamp et al., (1994) *Biotechnol. J.* 12:272-275; Rober et al., (1996) *Planta* 199:528-536). **Synthesis of fructan** in these non-fructan-storing plants was demonstrated, but accumulation was often very low and in tissues where high levels of fructan were reported to have a detrimental effect on plant development.

Brief Summary Text - BSTX:

The two classes of FTFs also differ in their affinity for sucrose, the sole substrate. Jerusalem artichoke sucrose-sucrose-**fructosyltransferase** (SST) has a K_m for sucrose reported to be approximately 100 mM (Koops, A. and Jonker, H., (1994) *J. Exp. Bot.* 45:1623-1631). By contrast, the bacterial enzyme has a much lower K_m of approximately 20 mM (Chambert, R., and Petit-Glatron, M. (1991) *Biochem. J.* 279:35-41). This difference may have a critical effect on **fructan synthesis**, resulting in higher or lower levels of accumulation, depending on the concentration of sucrose in the cell. The fundamental differences between FTF enzymes prevents meaningful predictions regarding the outcome of expression of plant genes in transgenic tissue, based on expression of bacterial FTF genes.

Brief Summary Text - BSTX:

Predicting whether or not fructan would accumulate in a transgenic line containing the plant-derived FTF genes could be significantly enhanced if a greater understanding of the fructan metabolic pathway in native fructan-storing plants existed. The currently accepted model for **fructan synthesis** in plants suggests that synthesis is a two step reaction. The initial reaction involves the enzyme sucrose-sucrose-**fructosyltransferase** (SST). SST catalyzes the synthesis of a trisaccharide from two sucrose residues. The second step, chain elongation, is carried out by the enzyme fructan-fructan-**fructosyltransferase** (FFT), (Edelman J., and Jefford T. (1968) *New Phytol.* 67:517-531. The model has been applied to all fructan-storing plants (ca 45000 species). However, it is based largely on data from a single species, *Helianthus tuberosus*, and has undergone several revisions. A recent study demonstrates that the SST can act alone in **producing long chain fructan** (Van der Ende, W. and Van Laere, A., (1996) *J. Exp. Bot.* 47:1797-1803). Thus, additional revisions in the model are necessary and suggests that there is only a rudimentary knowledge of **fructan synthesis** in plants.

Brief Summary Text - BSTX:

This invention discloses a method for producing fructose polymers of various lengths through expression of plant-derived FTF genes in a transgenic monocot species. More specifically, the invention describes a chimeric gene comprising a tissue specific promoter, operably linked to the coding sequence for a sucrose-sucrose-**fructosyltransferase** gene (SST; EC 2.4.1.99) such that said

chimeric gene is capable of transforming a monocot plant cell resulting in **production of fructan** with no deleterious effect on the said plant cell.

Brief Summary Text - BSTX:

The invention further describes a chimeric gene comprising a tissue specific promoter, operably linked to the coding sequence for a fructan-fructan-**fructosyltransferase** gene (FFT; EC 2.4.1.100) such that said chimeric gene is capable of transforming a transformed plant cell (harboring a chimeric gene comprising a tissue specific promoter, operably linked to the coding sequence for a sucrose-sucrose-**fructosyltransferase** gene (SST; EC 2.4.1.99)) resulting in **production of fructan**, with no deleterious effect on the said plant cell.

Brief Summary Text - BSTX:

The present invention is not limited to naturally occurring **fructosyl-transferases** but may equally well be performed by using modified versions thereof. Modifications may influence the activity of the **fructosyltransferase** in such a way that, for example, the degree of polymerization or the structure of the **fructan produced** is altered. Furthermore, according to the present invention a single **fructosyltransferase** gene or a combination of **fructosyltransferase** genes of plant origin may be used.

US-PAT-NO: 6326001

DOCUMENT-IDENTIFIER: US 6326001 B1

TITLE: Recombinant vaccine for diseases caused by encapsulated organisms

DATE-ISSUED: December 4, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|------------|-------|----------|---------|
| Inzana; Thomas J. | Blacksburg | VA | N/A | N/A |
| Ward; Christine | Irving | TX | N/A | N/A |

APPL-NO: 09/ 115824

DATE FILED: July 15, 1998

PARENT-CASE:

RELATED APPLICATION This application is a continuation of U.S. patent application Ser. No: 08/673,814, filed of Jun. 27, 1996, the complete contents of that application is herein incorporated by reference.

US-CL-CURRENT: 424/93.4; 424/93.2 ; 435/252.3 ; 435/440 ; 435/471

ABSTRACT:

Vaccines for diseases caused by normally encapsulated organisms are produced by genetically modifying those organisms by deleting the genes encoding for capsule synthesis or a portion thereof sufficient to produce non-capsulated mutants of the organisms. As an example, a live, attenuated strain of *Actinobacillus pleuropneumoniae* genetically modified with a large deletion in a chromosomal regions of the DNA which encodes for capsule synthesis is a safe and effective vaccine against swine pleuropneumonia.

5 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

Detailed Description Text - DETX:

The sucrose sensitivity of J45-100 was examined to determine whether the *sacRB* sequences could function as a counterselectable marker in *A. pleuropneumoniae* and subsequently induce the excision of the *nptI-sacRB* cartridge from the

J45-100 chromosome. Broth-grown J45-100 grew very heavily when plated directly or when diluted and then plated on TSY-N or Luria-Bertani (to which 5 μ g/ml NAD was added) medium containing 5% or 8% sucrose. The presence of the sacRB sequences in the chromosome of J45-100 was verified by Southern blotting. These results suggested that either the sacRB marker was not expressed in *A. pleuropneumoniae* or possibly that the levan product formed by the sacRB levansucrase in the presence of sucrose was not toxic to J45-100.

US-PAT-NO: 6255562

DOCUMENT-IDENTIFIER: US 6255562 B1

TITLE: Process for producing transgenic inulin-generating plants

DATE-ISSUED: July 3, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|--------|-------|----------|---------|
| Heyer; Arnd G. | Berlin | N/A | N/A | DE |
| Wendenburg; Regina | Berlin | N/A | N/A | DE |

APPL-NO: 09/ 180143

DATE FILED: November 3, 1998

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|------------|-------------|
| DE | 196 17 687 | May 3, 1996 |

PCT-DATA:

APPL-NO: PCT/EP97/02195
DATE-FILED: April 29, 1997
PUB-NO: WO97/42331
PUB-DATE: Nov 13, 1997
371-DATE: Nov 3, 1998
102(E)-DATE: Nov 3, 1998

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/252.3 ; 435/320.1 ; 435/412
; 435/417 ; 435/419 ; 435/468 ; 435/69.7 ; 435/69.8 ; 800/278 ; 800/287
; 800/288 ; 800/317.2 ; 800/320 ; 800/320.1 ; 800/320.2 ; 800/320.3

ABSTRACT:

The present invention relates to a process for preparing recombinantly modified, inulin-producing plants, to the DNA sequences which are used in this context and to the transformed plants which are obtained.

27 Claims, 6 Drawing figures

Exemplary Claim Number: 1,8,14

Number of Drawing Sheets: 6

----- KWIC -----

Brief Summary Text - BSTX:

Inulin, which is a .beta.-2-1-linked polyfructan, can be detected as a storage carbohydrate in some dicotyledonous higher plants and is present at a molecular weight of 5-50 kD. In addition, among the bacteria, some Gram-positive and Gram-negative bacterial species are known to synthesize a related fructan polymer, i.e. the .beta.-2-6-linked levan, using so-called levan sucrases. Polyfructans which are formed in bacteria exhibit substantially higher molecular weights of up to 2000 kD. At present, only one Gram-positive bacterium, i.e. Streptococcus mutans, has been described which uses an ftf (fructosyltransferase) gene to form inulin on the basis of sucrose (Shiroza and Kuramitsu, J. Bacteriology (1988) 170, 810 to 816).

Detailed Description Text - DETX:

However, the invention also relates to all other modifications of a fructosyltransferase gene, provided the gene product which is formed can produce high molecular weight inulin in plants.

Detailed Description Text - DETX:

In one embodiment, the invention relates to a modified fructosyltransferase gene which exhibits a signal sequence which encodes a signal peptide for uptake of the modified gene into the endoplasmic reticulum of a eukaryotic cell. The invention consequently envisages that a modified gene of the invention can be provided with signal sequences which permit location of the gene product in particular compartments of the cell. The signal sequence of a patatin gene, in particular of patatin gene B33, preferably from potato, is particularly preferred in accordance with the invention. As has been explained, the signal sequence can be fused onto the modified fructosyltransferase gene in addition to a modification of the fructosyltransferase gene which has already taken place, or the signal sequence is fused directly to the fructosyltransferase gene whose aminoterminal region has been truncated. Consequently, in both the preferred embodiments, it is envisaged, according to the invention, that the aminoterminal region of the fructosyltransferase gene is replaced with at least a part of the patatin gene and that, where appropriate, sequences from other genes, for example the lacZ gene or the cpy gene, are also additionally present in the aminoterminal region of the modified fructosyltransferase gene. Using the signal sequence, from the aminoterminal region of the patatin B33 gene, which encodes the signal peptide for uptake into the endoplasmic reticulum achieves translocation of the gene product into the apoplastic space. As a result, the synthesis of the high molecular weight inulin is carried out at this site, which means that specific changes in the carbohydrate composition of the transgenic plant can be effected. Naturally, other signal sequences can also be used in accordance with the invention. Thus, signal sequences which encode signal peptides which lead to the uptake of a protein into the endoplasmic reticulum, and which can be detected by the fact that they can be identified in the precursor proteins but not in processed, mature proteins, are particularly suitable. Thus, as is known, signal peptides are proteolytically removed during the uptake into the endoplasmic reticulum. In a further preferred embodiment, the invention provides that the modified

fructosyltransferase gene is fused to, or exhibits, a signal sequence which encodes a signal peptide for uptake into the endoplasmic reticulum of a eukaryotic cell and subsequent guidance into the vacuole. The use of a signal peptide for locating the gene product in a vacuole is advantageous insofar as this can also bring about specific changes in the carbohydrate composition of the resulting transgenic plants. For example, use can be made, according to the invention, of signal peptides for locating barley lectin in the vacuole (Raikhel and Lemer, Dev Genet (1991) 12, 255-260), signal sequences which encode 43 amino acids in the aminoterminal region of the mature bean phytohemagglutinin (Tague et al., Plant cell (1990) 2, 533-546) and signal sequences from a potato patatin gene.

Detailed Description Text - DETX:

The invention also relates to a vector, in particular a plasmid, which contains a modified fructosyltransferase gene. In particular, the invention relates to a vector or a plasmid which contains a modified fructosyltransferase gene which is under the control of a promoter which is active in plants, in particular an organ-specific promoter. As is known, sucrose is the substrate for fructosyltransferase, which means that it is advantageous to produce high molecular weight inulin particularly in those plant tissues or plant organs which store large quantities of sucrose. These include, for example, the beet of sugar beet or the stem of sugar cane. According to the invention, expression of the modified fructosyltransferase gene in these organs can be achieved by using tissue-specific promoters. For example, the B33 promoter of the potato B33 gene can be used to achieve organ-specific expression in potato tubers or beets of sugar beets.

Detailed Description Text - DETX:

The invention also relates to plants which contain at least one cell, preferably, however, a large number of cells, which exhibit(s) the novel fructosyltransferase gene or vectors or plasmids which contain this gene and as a result produce(s) high molecular weight inulin. The invention consequently makes it possible to provide plants of a very wide variety of species, genera, families, orders and classes which are able to produce high molecular weight inulin because of the modified fructosyltransferase gene which has been introduced into them. Since the known plants are not able to produce high molecular weight inulin, it is readily possible to demonstrate, for example by means of antibody tests, that the novel process has been successfully carried out. As compared with the little known inulin-producing plants, the advantages are gained that it is possible to specifically locate the inulin which is formed and that, in addition, an increase is achieved in the rate of expression and consequently in the quantity of inulin formed. Furthermore, the novel inulin which is produced by the gene, according to the invention, of bacterial origin exhibits a higher molecular weight than does plant inulin. In this case, too, successful transformation with the novel sequences can be demonstrated, for example, by means of compartment-specific antibody tests which can, where appropriate, be quantified.

Detailed Description Text - DETX:

The invention also relates to a process for preparing the above-mentioned plants, which process comprises the transformation of one or more plant cells with a vector or a plasmid of the invention, the integration of the modified **fructosyltransferase** gene, which is contained in this vector or plasmid, into the genome of the plant cell(s), and the regeneration of the plant cell(s) into intact, transformed, high molecular weight **inulin-producing** plants.

US-PAT-NO: 6180407

DOCUMENT-IDENTIFIER: US 6180407 B1

TITLE: Cloning and/or sequencing vector

DATE-ISSUED: January 30, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|----------|-------|----------|---------|
| Bernard; Philippe | Brussels | N/A | N/A | BE |
| Gabant; Philippe | Brussels | N/A | N/A | BE |

APPL-NO: 09/ 225152

DATE FILED: January 4, 1998

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of Application Ser. No. 08/379,614 filed Jul. 20, 1995, now U.S. Pat. No. 5,910,438, which is the U.S. National Phase under 35 U.S.C. Section 371 of International Application PCT/BE93/00051, filed Aug. 2, 1993 which claims priority of Application BE 09200696, filed Jul. 31, 1992.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|---------------|
| BE | 09200696 | July 31, 1992 |

US-CL-CURRENT: 435/471; 435/252.3 ; 435/252.33 ; 435/320.1 ; 435/480 ; 435/488

ABSTRACT:

A cloning and/or sequencing vector enables recombinant clones to be selected directly. The vector encodes a fusion protein which includes a protein poison.

14 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Brief Summary Text - BSTX:

Since the gene sacB encodes levan sucrase, which catalyses the hydrolysis of sucrose into products which are toxic for E. coli, direct selection of the

mutants which incorporate a recombinant plasmid is effected on a culture medium containing sucrose. Since the levan sucrase is toxic, even in the absence of sucrose, it is essential, consequently, to repress its synthesis if one wishes to obtain a large number of plasmid copies in the bacterial cytoplasm.

US-PAT-NO: 6147280

DOCUMENT-IDENTIFIER: US 6147280 A

TITLE: Production of oligosaccharides in transgenic plants

DATE-ISSUED: November 14, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|------------|-------|----------|---------|
| Smeeckens; Josephus | Driebergen | N/A | N/A | NL |
| Christianus Maria | De Meern | N/A | N/A | NL |
| Ebskamp; Michael | Utrecht | N/A | N/A | NL |
| Johannes Marcus | Den Dolder | N/A | N/A | NL |
| Geerts; Hendrikus | | | | |
| Andrianus Maria | | | | |
| Weisbeek; Petrus | | | | |
| Jacobus | | | | |

APPL-NO: 09/ 019385

DATE FILED: February 5, 1998

PARENT-CASE:

CROSS REFERENCE TO RELATED APPLICATIONS This application is a continuation of prior application Ser. No. 08/479,470, filed Jun. 7, 1999, now abandoned, which application is incorporated by reference herein.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| NL | 9401140 | July 8, 1994 |
| NL | 1000064 | April 5, 1995 |

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/320.1 ; 435/419 ; 435/440 ; 435/468 ; 435/69.7 ; 435/69.8 ; 536/23.2 ; 536/23.4 ; 536/23.6 ; 536/23.7 ; 800/288 ; 800/298

ABSTRACT:

The invention relates to a method for producing oligosaccharides, comprising selecting a gene which codes for an enzyme which is capable of converting sucrose into an oligosaccharide; linking the gene to suitable transcription-initiation and transcription-termination signals in order to provide an expression construct; transforming a suitable plant cell with the expression construct; transforming a suitable plant cell with the expression construct; regenerating a transgenic plant from the transformed plant cell; culturing the transgenic plant under conditions enabling the expression and activity of the enzyme; and isolating the oligosaccharides from the transgenic plant. The invention further relates to the product obtained by means of the

method and to the use thereof, in addition to transgenic plants and parts thereof which are capable of producing oligosaccharides.

18 Claims, 22 Drawing figures

Exemplary Claim Number: 1,12

Number of Drawing Sheets: 15

----- KWIC -----

Other Reference Publication - OREF:

Yamamoto, S. et al. "The Mode of Synthesis of Levan by Bacillus Subtilis Levansucrase", Agricultural and Biological Chemistry, vol. 49 No. 2, pp. 343-349 (Feb., 1985).

US-PAT-NO: 6110712

DOCUMENT-IDENTIFIER: US 6110712 A

TITLE: Cellulose-producing bacteria

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------|-------|----------|---------|
| Tsuchida; Takayasu | Kawasaki | N/A | N/A | JP |
| Tonouchi; Naoto | Kawasaki | N/A | N/A | JP |
| Seto; Akira | Kawasaki | N/A | N/A | JP |
| Kojima; Yukiko | Kawasaki | N/A | N/A | JP |
| Matsuoka; Masanobu | Kawasaki | N/A | N/A | JP |
| Yoshinaga; Fumihiko | Kawasaki | N/A | N/A | JP |

APPL-NO: 09/ 274470

DATE FILED: March 23, 1999

PARENT-CASE:

This application is a divisional of application Ser. No. 08/973,757 filed on Feb. 3, 1998, now U.S. Pat. No. 5,962,278 issued Oct. 5, 1999.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|----------------|
| JP | 8-123951 | April 23, 1996 |
| JP | 8-149763 | May 22, 1996 |
| JP | 8-174393 | June 14, 1996 |

PCT-DATA:

APPL-NO: PCT/JP87/00514
DATE-FILED: February 24, 1997
PUB-NO:
PUB-DATE:
371-DATE: Feb 3, 1998
102(E)-DATE: Feb 3, 1998

US-CL-CURRENT: 435/101; 435/252.1 ; 435/823

ABSTRACT:

This invention relates to a microorganism that is capable of producing a cellulosic product (referred to hereinafter as a "cellulose-producing bacterium") and belongs to a novel subspecies which is substantially negative or very slightly positive in oxidation of acetates and lactates. This invention also relates to novel saccharide analog-resistant strains, amino acid analog-resistant strains and levan sucrase-defective strains. Further, this

invention relates to a method for the production of cellulosic material (bacterial cellulose:"BC"), which comprises culturing these novel bacteria and to bacterial cellulose which may be thus obtained. A larger amount of bacterial cellulose may be produced by culturing *Acetobacter xylinum* subsp. nonacetoxidans, the present resistant strains and the levan sucrase-defective strains, which have been derived and bred from the cellulose-producing bacteria, than by culturing the BPR 2001 strain in the medium containing especially sucrose or glucose as carbon sources.

4 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Mutants include a **levan sucrase-defective mutant in which the production of levan** is suppressed.

Brief Summary Text - BSTX:

Levan **sucrase** (EC2.4.1.10) is known to decompose and metabolize sucrose. This enzyme has two activities, (1) hydrolysis activity of sucrose into glucose and fructose, and (2) transfructosylation activity to **produce glucose and levan** from sucrose. The latter activity is not preferred in terms of BC **production since it will produce levan as a by-product**. It will be very advantageous in the **production and purification of BC, if the accumulation of levan** is suppressed. The present inventors have already disclosed in the Japanese Patent Application Hei 7 (1995)-252021 that by deriving the levan **sucrase**-defective strain and incorporating the gene of an extracellular invertase or a **levan sucrase with a reduced levan productivity into said strain, the accumulation of levan will be reduced and the cellulose may be efficiently produced**.

Detailed Description Text - DETX:

The 757-3-5-11 strain was subjected to the same mutagenesis treatment as above, and inoculated on a plate containing the minimum medium shown in Table 10 using sucrose instead of glucose. The small non-mucoidal colonies **producing no levan** were selected as candidate strains. Further, the two strains (LD-1 and LD-2) producing cellulose well in the Glc--CSL medium (Table 11) were selected from the above candidates. Table 24 shows the production of cellulose and polysaccharide as by-product by these two strains in CSL-Glc and CSL-Suc media (Table 5). No activity of levan **sucrase** was observed in these two strains by the usual method (H. Yanase et al., Biosci. Biotech. Biochem., Vol. 55 pp.1383-1390, 1991), confirming that these two strains are levan **sucrase**-defective strains.

Detailed Description Text - DETX:

The levan sucrase-defective strain, LD-2 was transformed with the plasmid pSAZE3S containing the exocellular invertase gene and its secretion-accelerating gene of Zymomonas mobilis strain disclosed in the Japanese Patent Application Hei 7 (1995)-252021, or the plasmid pSARH containing the gene of Bacillus subtilis encoding a levan sucrase with its reduced activity for levan formation. The production of cellulose by these transformants in the CSL-Suc medium (Table 5) is shown in Table 25. The introduction of the above genes into the levan sucrase-defective strain has remarkably reduced the amount of levan as by-product in the production of cellulose from sucrose.

Claims Text - CLTX:

3. A method for producing a cellulosic product, which comprises culturing a levan sucrase-defective strain of Acetobacter xylinum subsp. nonacetoxidans, that is negative or very slightly positive in oxidation of acetates and lactates, and isolating a cellulosic product.

US-PAT-NO: 6086894

DOCUMENT-IDENTIFIER: US 6086894 A

TITLE: Recombinant vaccine for diseases caused by encapsulated organisms

DATE-ISSUED: July 11, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|------------|-------|----------|---------|
| Inzana; Thomas J. | Blacksburg | VA | N/A | N/A |
| Ward; Christine | Irving | TX | N/A | N/A |

APPL-NO: 08/ 673814

DATE FILED: June 27, 1996

US-CL-CURRENT: 424/235.1; 424/256.1 ; 424/825 ; 424/93.2 ; 435/243 ; 435/245 ; 435/252.3 ; 435/440 ; 435/471

ABSTRACT:

Vaccines for diseases caused by normally encapsulated organisms are produced by genetically modifying those organisms by deleting the genes encoding for capsule synthesis or a portion thereof sufficient to produce non-capsulated mutants of the organisms. As an example, a live, attenuated strain of *Actinobacillus pleuropneumoniae* genetically modified with a large deletion in a chromosomal regions of the DNA which encodes for capsule synthesis is a safe and effective vaccine against swine pleuropneumonia.

4 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

----- KWIC -----

Detailed Description Text - DETX:

The sucrose sensitivity of J45-100 was examined to determine whether the *sacRB* sequences could function as a counterselectable marker in *A. pleuropneumoniae* and subsequently induce the excision of the *nptI-sacRB* cartridge from the J45-100 chromosome. Broth-grown J45-100 grew very heavily when plated directly or when diluted and then plated on TSY-N or Luria-Bertani (to which 5 Ilg/ml NAD was added) medium containing 5% or 8% sucrose. The presence of the *sacRB* sequences in the chromosome of J45-100 was verified by Southern blotting. These results suggested that either the *sacRB* marker was not expressed in *A.*

pleuropneumoniae or possibly that the levan product formed by the sacRB levansucrase in the presence of sucrose was not toxic to J45-100.

US-PAT-NO: 6071725

DOCUMENT-IDENTIFIER: US 6071725 A

TITLE: Vectors expressing ice nucleation protein fusions for cell surface anchoring of foreign proteins

DATE-ISSUED: June 6, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------|-----------|-------|----------|---------|
| Pan; Jae Gu | Taejon-si | N/A | N/A | KR |
| Jung; Heung Chae | Taejon-si | N/A | N/A | KR |
| Park; Seung Hwan | Taejon-si | N/A | N/A | KR |
| Han; Moon Hi | Taejon-si | N/A | N/A | KR |
| Park; Young Hoon | Taejon-si | N/A | N/A | KR |

APPL-NO: 08/ 973180

DATE FILED: December 2, 1997

PARENT-CASE:

This application is a national stage application of PCT/KR97/00057 filed on Apr. 2, 1997, which claims priority from Korean Application Ser. No. 96-9921 filed on Apr. 2, 1996.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| KR | 96-9921 | April 2, 1996 |

PCT-DATA:

APPL-NO: PCT/KR97/00057
DATE-FILED: April 2, 1997
PUB-NO: WO97/37025
PUB-DATE: Oct 9, 1997
371-DATE: Dec 2, 1997
102(E)-DATE: Dec 2, 1997

US-CL-CURRENT: 435/69.7; 435/476 ; 435/488 ; 435/69.6 ; 435/69.8 ; 435/71.1 ; 435/71.2

ABSTRACT:

The present invention relates to surface anchoring vectors, a method for preparation of foreign proteins onto a cell surface and use thereof, which uses outer cell membrane protein, ice nucleation protein (NIP) derived from *Pseudomonas syringae*, a gram-negative bacterium.

24 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Brief Summary Text - BSTX:

In addition, the present invention can provide uses of foreign proteins expressed onto a cell surface, which comprises effective production of antibodies and antigens and production of libraries for screening antigens, binding or adsorbent proteins, physiological activators and the like. For example levansucrase expressed onto the cell surface can be utilized to produce levan efficiently.

Detailed Description Text - DETX:

In addition, levansucrase expressed onto the cell surface can be used to produce levan from sucrose conveniently and efficiently (see FIG. 9.).

Detailed Description Text - DETX:

Especially levansucrase expressed onto the cell surface is very useful to produce levan from sucrose and the process of the present invention can be used for the bioconversion efficiently.

US-PAT-NO: 6057494

DOCUMENT-IDENTIFIER: US 6057494 A

TITLE: DNA sequences encoding carbohydrate polymer synthesizing enzymes and method for producing transgenic plants

DATE-ISSUED: May 2, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP | CODE | COUNTRY |
|------------------------|------------|-------|-----|------|---------|
| Koops; Andries Juriaan | Wageningen | | N/A | N/A | NL |
| van der Meer; Ingrid | Wageningen | | N/A | N/A | NL |
| Maria | Wageningen | N/A | N/A | NL | |
| Van Tunen; Arjen | | | | | |
| Johannus | | | | | |

APPL-NO: 08/ 860091

DATE FILED: August 25, 1997

PARENT-CASE:

This application is a 371 of PCT/NL96/00012 filed Jan. 8, 1996.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-----------------|
| EP | 95200015 | January 6, 1995 |
| EP | 95200762 | March 27, 1995 |

PCT-DATA:

APPL-NO: PCT/NL96/00012
DATE-FILED: January 8, 1996
PUB-NO: WO96/21023
PUB-DATE: Jul 11, 1996
371-DATE: Aug 25, 1997
102(E)-DATE: Aug 25, 1997

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/419 ; 435/468 ; 435/471
; 536/23.6 ; 536/24.5 ; 800/286 ; 800/298

ABSTRACT:

A DNA fragment is disclosed having a nucleotide sequence SEQ ID NO. 1 as shown in FIG. 4A or a homologous sequence having a similarity of at least 70% encoding 1-sucrose:sucrose fructosyltransferase. Another DNA fragment that is disclosed is a DNA fragment having a nucleotide sequence SEQ ID NO. 3 as shown in FIG. 4B or a homologous sequence having a similarity of at least 70% encoding 1-fructan:fructan fructosyltransferase. This invention also discloses a recombinant DNA comprising one or more of said DNA fragments, or comprising

said DNA fragment(s) in the inverted orientation. Transformed organisms showing a modified fructan profile can be produced using said fragments.

26 Claims, 9 Drawing figures

Exemplary Claim Number: 1,2,10,11

Number of Drawing Sheets: 12

----- KWIC -----

Abstract Text - ABTX:

A DNA fragment is disclosed having a nucleotide sequence SEQ ID NO. 1 as shown in FIG. 4A or a homologous sequence having a similarity of at least 70% encoding 1-sucrose:sucrose fructosyltransferase. Another DNA fragment that is disclosed is a DNA fragment having a nucleotide sequence SEQ ID NO. 3 as shown in FIG. 4B or a homologous sequence having a similarity of at least 70% encoding 1-fructan:fructan fructosyltransferase. This invention also discloses a recombinant DNA comprising one or more of said DNA fragments, or comprising said DNA fragment(s) in the inverted orientation. Transformed organisms showing a modified fructan profile can be produced using said fragments.

Brief Summary Text - BSTX:

In bacteria, examples of fructan synthesizing bacteria are Streptococcus mutans and Bacillus subtilis, the biosynthesis of fructans from sucrose is catalysed by only one enzyme: levansucrase (EC 2.4.1.10) in B. subtilis (Dedonder 1966) and levansucrase, but also called fructosyltransferase, (FTF, EC 2.4.1.10) in S. mutans (Carlsson, 1970). Bacterial fructan synthesis proceeds via the direct transfer of fructose from a donor-sucrose (G-F) to sucrose or other acceptor molecules according to the following reversible reaction:

Brief Summary Text - BSTX:

Bacterial DNA sequences encoding FTF in S. mutans and levansucrase in B. subtilis are already described in the literature (Sato and Kuramitsu, 1986; Steinmetz et al. 1985). Bacterial genes from several sources were used to transform specific host plants which normally cannot synthesize fructans, thereby inducing fructan synthesis (see for example: Van der Meer et al., 1994; Ebskamp et al., 1994). A method to enhance the solid content of tomato fruits, using the levansucrase gene from B. subtilis and the dextransucrase gene from Leuconostoc mesenteroides is described in application WO 89/12386. A method to modify the fructan pattern in plants which normally cannot synthesize fructans, using the levansucrase-encoding ftf gene from S. mutans and the levansucrase-encoding SacB gene from B. subtilis is described in applications NL A 9300646 and WO 94/14970. The use of a levansucrase-encoding DNA sequence from Erwinia amylovora, which after integration in the host plant genome leads

to the synthesis of levans, is described in DE 4227061 A1 and WO A 9404692. In all said applications, transgenic plants are described which are transformed with levansucrase genes from bacteria. Accordingly, these transgenic plants synthesize and accumulate fructans structurally comparable to those synthesized by the donor bacteria (Van der Meer et al., 1994; Ebskamp et al., 1994).

Brief Summary Text - BSTX:

The present application differs from said applications in that it is related to fructosyltransferase-encoding DNA sequences derived from plants. These enzymes are structurally different from bacterial enzymes since there is no significant homology at the amino acid level and DNA level. Besides, the mechanism of fructan biosynthesis in plants is essentially different from that in bacteria. In contrast to fructan biosynthesis in bacteria, the formation of fructans in plants is mediated by more than one enzyme. For example, in *Helianthus tuberosus* (the Jerusalem Artichoke), fructan biosynthesis is catalysed by two enzymes: sucrose:sucrose fructosyltransferase (SST, EC 2.4.1.99) and fructan:fructan fructosyltransferase (FFT, EC 2.4.1.100). The SST and FFT from *H. tuberosus* are involved in the synthesis of .beta.-2,1 linked fructans (inulin) and are therefore also designated as 1-SST and 1-FFT. 1-FFT has been purified from tubers of *H. tuberosus* (Luscher et al., 1993, Koops and Jonker, 1994). The purification of SST has proven more difficult

Detailed Description Text - DETX:

Sucrose:sucrose fructosyltransferase (SST): Plant-derived enzyme catalyzing the initial step of fructan synthesis (reaction 2). Enzyme can also be involved in the synthesis of oligofructans [G-(F).sub.n, 2.ltoreq.n.ltoreq.4] (reaction 3). In the present application, the designation SST can include either 1-SST, an SST form involved in the biosynthesis of oligofructans that has mostly the .beta.-2,1-fructosyl-fructose linkage; or 6-SST, an SST form involved in the biosynthesis of oligofructans that has mostly the .beta.-2,6-fructosyl-fructose linkage; or 1-SST and 6-SST.

Detailed Description Text - DETX:

Fructan:fructan fructosyltransferase (FFT). Plant-derived enzyme involved in the synthesis of fructans. Enzyme capable of catalyzing the synthesis of oligofructans and fructans of a higher degree of polymerization. FFT from *H. tuberosus* has overlapping activity with SST from *H. tuberosus* (reaction 3), but cannot catalyse the initial step of fructan synthesis (reaction 2). In the present application, the designation FFT can include either 1-FFT, an FFT form involved in the biosynthesis of oligofructans that has mostly the .beta.-2,1-fructosyl-fructose linkage; or 6-FFT, an FFT form involved in the biosynthesis of fructans that has mostly the .beta.-2,6-fructosyl-fructose linkage; or 1-FFT and 6-FFT.

US-PAT-NO: 6028249

DOCUMENT-IDENTIFIER: US 6028249 A

TITLE: DNA sequences which lead to the formation of levans, plasmids containing these sequences as well as a process for preparing transgenic plants

DATE-ISSUED: February 22, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------|--------------------|-------|----------|---------|
| Rober; Manuela | Berlin | N/A | N/A | DE |
| Geier; Gebhard | Heidelberg/Dossenh | N/A | N/A | DE |
| Geider; Klaus | eim | N/A | N/A | DE |
| | Sandhausen | | | |

APPL-NO: 08/ 943374

DATE FILED: October 3, 1997

PARENT-CASE:

This application is a division of application Ser. No. 08/381,936 filed on Feb. 9, 1995, now U.S. Pat. No. 5,792,923, which is a 371 of PCT/EP93/02110 filed Aug. 9, 1993.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|-----------|-----------------|
| DE | 42 27 061 | August 12, 1992 |

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/320.1 ; 435/468 ; 435/69.1 ; 536/23.7 ; 800/287 ; 800/288 ; 800/298 ; 800/317.2 ; 800/317.3 ; 800/320 ; 800/320.1 ; 800/320.2 ; 800/320.3

ABSTRACT:

There are described DNA sequences, which lead to the formation of polyfructans (levans), plasmids containing theses DNA sequences, as well as a process using these plasmids for preparing transgenic plants with polyfructan (levan) expression.

12 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Brief Summary Text - BSTX:

Until now, genes for levan sucrase from *Bacillus amyloliquefaciens* (Tang et al. (1990) Gene 96, 89-93) and *Bacillus subtilis* (Steinmetz et al. (1985) Mol. Gen. Genetics 200, 220-228), have been described, which demonstrate relatively high homology with each other and both of which catalyse the synthesis of fructans of the levan type. Further a fructosyl transferase from *Streptococcus mutans* (Shiroza et al. (1988) J. Bacteriology 170, 810-816) has been described. This shows little homology to either levan sucraes from *Bacillus* spp. The fructan formed in *Streptococcus mutans* is of the inulin type.

Brief Summary Text - BSTX:

In WO 89/12386, there is described the possibility of producing carbohydrate polymers such as dextran or levan in transgenic plants, especially in the fruit of transgenic plants. To prepare these plants, the use of levan sucraes from *Aerobacter levanicum*, *Streptococcus salivarius* and *Bacillus subtilis* and the use of dextran sucraes from *Leuconostoc mesenteroides* have been described. Further the construction of chimeric genes is described which may be suitable for the expression of the levan sucrase from *Bacillus subtilis* as well as the dextran sucrase from *Leuconostoc mesenteroides* in transgenic plants. Also described is the preparation of transgenic plants containing these constructs. Further, the preparation of transgenic plants that contain these constructs are described. Whether polyfructans can actually be produced by the described process is not known. There is also described a series of processes for modifying the carbohydrate concentration and/or concentrating carbohydrate in transgenic plants by means of biotechnological methods. Thus, in view of the fact that increasing starch concentration and modification of the starch in physical and chemical respects is already known, then a modification of the carbohydrate content of potato plants by raising or lowering the ADP-glucose-pyrophosphorylase activity can be achieved (EP 455 316).

Brief Summary Text - BSTX:

Since sucrose represents the substrate for the levan sucrase, the production of polyfructans is especially advantageous in those organs which store large amounts of sucrose. Such organs are for example the roots of sugar beet or the stems of sugar cane. It is especially useful in genetically modified potatoes, which store sucrose in their tubers, through blocking of starch biosynthesis

US-PAT-NO: 6025542

DOCUMENT-IDENTIFIER: US 6025542 A

TITLE: Method for obtaining transgenic plants showing a modified fructan pattern

DATE-ISSUED: February 15, 2000

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|------------|-------|----------|---------|
| Smeeckens; Josephus | Driebergen | N/A | N/A | NL |
| Christianus Maria | De Meer | N/A | N/A | NL |
| Ebskamp; Michael | Den Dolder | N/A | N/A | NL |
| Johannes Marcus | | | | |
| Weisbeek; Petrus | | | | |
| Jacobus | | | | |

APPL-NO: 08/ 446834

DATE FILED: August 3, 1995

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-------------------|
| EP | 92204098 | December 28, 1992 |
| NL | 9300646 | April 15, 1993 |

PCT-DATA:

APPL-NO: PCT/NL93/00279
DATE-FILED: December 28, 1993
PUB-NO: WO94/14970
PUB-DATE: Jul 7, 1994
371-DATE: Aug 3, 1995
102(E)-DATE: Aug 3, 1995

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/320.1 ; 435/418 ; 435/419 ; 435/468 ; 435/69.7 ; 435/69.8 ; 536/23.2 ; 536/23.4 ; 536/23.6 ; 800/287 ; 800/288 ; 800/289 ; 800/298

ABSTRACT:

The present invention relates to a method for obtaining transgenic plants showing a modified fructan pattern as compared to non-transformed plants, comprising the steps of: a) preparing a DNA construct comprising one or more fructosyltransferase genes, or modified versions thereof, being operably linked to a promoter sequence active in plants and a terminator sequence active in plants; b) transforming a plant cell with the construct; and c) regenerating a transgenic plant from the transformed plant cell. Preferably, the 5' untranslated region of the fruct syltransferase gene is modified such that the expression of the fructosyltransferase is not negatively affected. The DNA

construct may also comprise a targeting sequence upstream from the **fructosyltransferase** gene for directing the **fructosyltransferase** to a specific plant tissue or plant cell compartment. The **fructosyltransferases produced in the transgenic plants change the fructan** pattern of the plants, thus leading to different plant performance due to altered sink-source relations and yield, increased tolerance for drought, cold or other stresses, higher dry matter content, better taste and storability, and improved nutritional value. The plants are also suitable for use as raw material for **fructan production**.

29 Claims, 9 Drawing figures

Exemplary Claim Number: 1,15

Number of Drawing Sheets: 9

----- KWIC -----

Abstract Text - ABTX:

The present invention relates to a method for obtaining transgenic plants showing a modified fructan pattern as compared to non-transformed plants, comprising the steps of: a) preparing a DNA construct comprising one or more **fructosyltransferase** genes, or modified versions thereof, being operably linked to a promoter sequence active in plants and a terminator sequence active in plants; b) transforming a plant cell with the construct; and c) regenerating a transgenic plant from the transformed plant cell. Preferably, the 5' untranslated region of the **fructosyltransferase** gene is modified such that the expression of the **fructosyltransferase** is not negatively affected. The DNA construct may also comprise a targeting sequence upstream from the **fructosyltransferase** gene for directing the **fructosyltransferase** to a specific plant tissue or plant cell compartment. The **fructosyltransferases produced in the transgenic plants change the fructan** pattern of the plants, thus leading to different plant performance due to altered sink-source relations and yield, increased tolerance for drought, cold or other stresses, higher dry matter content, better taste and storability, and improved nutritional value. The plants are also suitable for use as raw material for **fructan production**.

Brief Summary Text - BSTX:

The term "fructan pattern" refers to the distribution of the fructan in the different plant tissues and plant cell compartments such as cytosol, vacuole, apoplast etc. Some plants naturally do produce fructans whereas others do not. The **fructan pattern of a plant species not producing** fructans in the non-transformed plant may be changed by the introduction of a gene encoding a **fructosyltransferase**. The fructan distribution in a plant may also be changed by the redirection of the metabolic flow in plants or plant cells to certain plant or plant cell compartments.

Brief Summary Text - BSTX:

The fructosyltransferases of the present invention use sucrose as a substrate to synthesize high molecular weight fructans. Many micro-organisms contain fructosyltransferases which have the ability to produce fructans (often called levans) from sucrose (A. Fuchs 1959, Thesis, University of Leiden, Han 1989 Adv. Appl. Microbiol. 35, 171-194). These enzymes can transfer fructose moieties from sucrose to a fructan acceptor molecule to produce high molecular weight fructans. Since these fructan acceptor molecules are originally derived from sucrose they may still contain a terminal glucose molecule. The reaction mechanism is as follows (review see Han 1989 Adv. Appl. Microbiol. 35, 171-194):

Brief Summary Text - BSTX:

Two examples of fructosyltransferase producing bacteria are *Bacillus subtilis* (Steinmetz et al. 1985 Mol. Gen. Genet. 200, 220-228) and *Streptococcus mutans* (Shiroza, and Kuramitsu 1988 J. Bacteriol. 170, 810-816. In *B. subtilis* the *sacB* gene encodes the structural gene for levansucrase, a fructosyltransferase. This enzyme can convert sucrose into a fructose polymer with DP's which can easily exceed 10000. The linkage type found in this fructan produced by the levansucrase is mainly of the (2-6)-type with extensive (2-1)-branching.

Brief Summary Text - BSTX:

The present invention is not limited to naturally occurring fructosyltransferases but may equally well be performed by using modified versions thereof. Modifications may influence the activity of the fructosyltransferases in such a way that e.g. the degree of polymerization or the structure of the fructan produced is altered.

Detailed Description Text - DETX:

To create fructan-producing transgenic plants, genes encoding proteins capable of producing fructans were selected. One of these genes, levansucrase, encoded by the *sacB* gene of *Bacillus subtilis* (Steinmetz M. et al. Mol. Gen. Genet. 200:220-228(1985)), was used. This enzyme produces mainly branched fructans of the (2-6)-linkage type in the presence of sucrose.

Detailed Description Text - DETX:

Since sucrose in plant cells can accumulate in vacuoles, this is a preferred site for fructan production. To direct the levansucrase to the vacuole the targeting region of carboxypeptidase Y (Valls L. A. et al. Cell 48, 887-897 (1987)) was selected.

Detailed Description Text - DETX:

The fructans accumulating in these plants were further studied by isolation of larger quantities (Livingstone III D. A., Plant Physiol. 92, 767-769 (1990)). This fructan was analysed by sizing on a FPLC Superose 6HR 10/30 column (Pharmacia) and fructans were detected in the void volume indicating a degree of polymerisation of $>25,000$ fructose units. **Fructan produced** by *Bacillus subtilis* **levansucrase** similarly elutes in the void volume of the Superose column.

Detailed Description Text - DETX:

The purified fructans were also analysed by proton-NMR. Fructan isolated from transgenic plants showed no differences in peak pattern when compared with **fructan synthesised by levansucrase** from *Bacillus subtilis*.

Detailed Description Text - DETX:

To create **fructan-producing** plants, genes encoding proteins capable of producing fructans were selected. Another gene, **fructosyltransferase** encoded by the *ftf* gene of *Streptococcus mutans* (Shiroza T. and Kuramitsu H. K., J. Bacteriol. 170, 810-816 (1988)), was used. This enzyme produces mainly branched fructans of the (2-1)-linkage type in the presence of sucrose. Since sucrose in plant cells can accumulate in vacuoles, this is a preferred site for fructan accumulation. A gene encoding a protein capable of targeting itself to the vacuole was selected: carboxypeptidase Y (Valls L. A. et al., supra). From this gene the signal sequence and vacuolar targeting sequence were used as described in Example 1.

Detailed Description Text - DETX:

Screening of the transformants, using the TLC method as in Example 1, showed accumulation of fructan with the normal variation due to position effect. The fructans accumulating in these plants were further studied by isolation of larger quantities (Livingstone III D. A., supra). This fructan was analysed by sizing on a FPLC Superose 6HR 10/30 column (Pharmacia) and fructans were detected in the void volume indicating a degree of polymerisation of $>25,000$ fructose units. **Fructan produced** by *Streptococcus mutans* **fructosyltransferase** similarly elutes in the void volume of the Superose column. Similarly partial fructan degradation by acid degradation shows a characteristic pattern of hydrolysis products. The purified plant and bacterial fructans show identical degradation patterns on TLC. Full acid hydrolysis followed by HPLC analysis on an Aminex HPX87C column at 85.degree. C. using water as eluent shows this fructan to be composed of fructose.

Detailed Description Text - DETX:

To create plants which produce fructans, genes encoding proteins capable of producing fructans were selected. Another gene, **fructosyltransferase** encoded

by the ftf gene (Shiroza T. and Kuramitsu H. K., supra) and introduced in Example 2, was used. Since sucrose in plant cells is synthesized in the cyt plasm, the cytoplasm is a preferred site for fructan accumulation. Since nuclear-encoded proteins are made in the cytoplasm no targeting sequence is needed.

Other Reference Publication - OREF:

Yamamoto, S., et al. "The Mode of Synthesis of Levan by Bacillus Subtillis Levansucrase", Agricultural and Biological Chemistry, vol. 49, No. 2, pp. 343-349 (Feb., 1985).

US-PAT-NO: 5998177

DOCUMENT-IDENTIFIER: US 5998177 A

TITLE: Process for processing sucrose into glucose

DATE-ISSUED: December 7, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|----------|-------|----------|---------|
| Catani; Steven J. | Athens | GA | N/A | N/A |
| Roth; Stephen A. | Gladwyne | PA | N/A | N/A |
| McGuire; Edward J. | Furlong | PA | N/A | N/A |
| Navia; Juan L. | Athens | GA | N/A | N/A |

APPL-NO: 09/ 195680

DATE FILED: November 19, 1998

US-CL-CURRENT: 435/101; 435/105 ; 435/193 ; 435/97

ABSTRACT:

The present invention relates to a process of preparing commercial quantities of glucose from sucrose, a process of preparing commercial quantities of glucose and a branched fructan from sucrose, a reactor for practicing same.

12 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

Brief Summary Text - BSTX:

The present invention relates to a process of preparing commercial quantities of glucose from sucrose, and a reactor for practicing same. In particular, the present invention relates to a process for preparing glucose from sucrose by contacting sucrose with a .beta.-2,1-**fructosyltransferase** and a .beta.-2,6-**fructosyltransferase**, followed by isolating glucose and a branched **fructan, thereby enhancing production** efficiencies.

Brief Summary Text - BSTX:

The objects above may also be accomplished with a process for preparing glucose, by contacting sucrose with a .beta.-2,1-fructosyltransferase and a .beta.-2,6-fructosyltransferase in a reactor to produce reaction products comprising glucose and a branched fructan, followed by isolating glucose and a branched fructan.

Brief Summary Text - BSTX:

In another embodiment, a process for preparing glucose may be achieved, by contacting sucrose sequentially first with a chain extending .beta.-2,1-fructosyltransferase and second with a branching .beta.-2,6-fructosyltransferase in a reactor to produce reaction products comprising glucose and a branched fructan, which is nearly depleted of sucrose.

Detailed Description Text - DETX:

In one embodiment, a linear inulin fructan comprising .beta.-2,1-linkages is formed under the action of a chain-extending .beta.-2,1-fructosyltransferase, followed by branching with fructose units under the action of a branching .beta.-2,6-fructosyltransferase. Sucrose would be the fructose donor for both fructosyltransferases. Sucrose and the terminal fructose of a fructan would be acceptors for the .beta.-2,1-fructosyltransferase, while a linear fructan chain would be an acceptor for a .beta.-2,6-fructosyltransferase. The linear inulin fructan comprising .beta.-2,1-linkages may be formed in one or more stages, the use of a one stage process favoring the formation of a greater number of inulin chains of low DP, the use of a process comprising more than one stage favoring the production of fewer but longer inulin chains (e.g., higher DPs). In order to form longer inulin chains, fructan formation is induced with a portion of the sucrose to be converted, followed by addition of the remaining sucrose. Such a procedure for forming extended linear fructans (e.g higher DPs) is described by Catani et al. in co-pending U.S. Ser. No. 09/019,709, filed on Feb. 6, 1998, the entire contents of which are hereby incorporated by reference.

Detailed Description Text - DETX:

In another embodiment, a linear levan fructan comprising .beta.-2,6-linkages is formed under the action of a .beta.-2,6-fructosyltransferase, followed by branching with fructose units under the action of a .beta.-2,1-fructosyltransferase. Sucrose would be the fructose donor for both fructosyltransferases. Sucrose and the terminal fructose of a fructan would be an acceptor for the .beta.-2,6-fructosyltransferase while a linear fructan chain would be an acceptor for a .beta.-2,1-fructosyltransferase. The linear levan fructan comprising .beta.-2,6-linkages may be formed in one or more stages, the use of a one stage process favoring the formation of a greater number of levan chains of low DP, the use of a process comprising more than one stage favoring the production of fewer but longer levan chains (e.g., higher DPs). In order to form longer levan chains, fructan formation is induced with a portion of the sucrose to be converted, followed by addition of the remaining

sucrose. Such a procedure for forming extended linear levan fructans is as described for the preparation of extended linear inulin fructans.

Detailed Description Text - DETX:

Now referring to FIG. 2, where 1 depicts a first reactor, 2 and 11 depict inlets for sucrose, 3 and 8 depict outlets for glucose, 4 depicts an outlet for a linear fructan, 5 and 10 depict separators which are permeable to glucose but not permeable to sucrose, a fructosyltransferase or a higher fructan, 6 depicts a second reactor, 7 depicts an inlet for a linear fructan and 9 depicts an outlet for a branched fructan. Two reactors are used, each partitioned with separators 5 and 10 which are permeable to glucose but impermeable to sucrose, fructosyltransferases or to linear or branched fructans. In the first reactor 1, the concentration of sucrose is such as to provide for the synthesis of linear .beta.-2,1-inulin fructans, the product then being transferred to the second reactor 6 via inlet for a linear fructan. In a preferred embodiment, either the outlet for linear fructan 4 or the inlet for linear fructan 7 does not permit passage of an active fructosyltransferase. This may be accomplished by equipping either the outlet 4 or the inlet 7 with a membrane which does not permit the passage of a fructosyltransferase. Alternatively, either the outlet 4 or the inlet 7 may be equipped with a deactivation zone from fructosyltransferase, such as by heating for a sufficient time and temperature, typically about 65 to 95.degree. C., preferably from about 70 to 90.degree., even more preferably about 85.degree., for about one minute. In the second reactor 6, a .beta.-2,6-fructosyltransferase is contained in a portion of a second reactor 6 and a linear fructan is reacted with sucrose. Glucose is permitted to pass through separator 10 and is removed via glucose outlet 8. During the separation of glucose, the remaining materials may be recycled to reactor portion 6. The branched fructan may be removed via branched fructan outlet 9. The outlet for branched fructan 9, may be equipped with a separator (not shown) which permits the passage of branched fructans, but does not permit passage of sucrose, glucose or fructosyltransferases.

Detailed Description Text - DETX:

The process of the present invention is preferably conducted in a reactor suitable for making commercial quantities of branched fructan. Preferably the reactor comprises one or more inlets for introducing sucrose and/or the fructosyltransferase and a means for isolating commercial quantities of branched fructan from the reactor. The reactor may comprise multiple vessels, as illustrated in FIGS. 2 and 2a, functioning as a reactor system.

US-PAT-NO: 5986173

DOCUMENT-IDENTIFIER: US 5986173 A

TITLE: Method for obtaining transgenic plants showing a modified fructan pattern

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|------------|-------|----------|---------|
| Smeebens; Josephus | Driebergen | N/A | N/A | NL |
| Christianus Maria | De Meern | N/A | N/A | NL |
| Ebskamp; Michael | Den Dolder | N/A | N/A | NL |
| Johannes Marcus | | | | |
| Weisbeek; Petrus | | | | |
| Jacobus | | | | |

APPL-NO: 09/ 059484

DATE FILED: April 13, 1998

PARENT-CASE:

This application is a continuation of copending U.S. application Ser. No. 08/446,834, having a filing date of Aug. 3, 1995, which is a 371 of prior international application serial number PCT/NL93/00279, filed Dec. 28, 1993, which claims priority of Netherlands Patent No. 9300646 filed Apr. 15, 1993 which claims priority of European Patent No. 92204098.5 filed Dec. 28, 1992, priority from the filing date of which is hereby claimed under 35 U.S.C. .sctn..sctn. 119 and 120.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-------------------|
| EP | 92204098 | December 28, 1992 |
| NL | 9300646 | April 15, 1993 |

US-CL-CURRENT: 800/284; 435/101 ; 435/193 ; 435/468 ; 435/69.7 ; 435/69.8 ; 536/23.4 ; 536/23.6 ; 536/23.7 ; 536/24.1 ; 800/287 ; 800/288 ; 800/298 ; 800/320 ; 800/320.1

ABSTRACT:

The present invention relates to a method for obtaining transgenic plants showing a modified fructan pattern as compared to non-transformed plants, comprising the steps of: a) preparing a DNA construct comprising one or more fructosyltransferase genes, or modified versions thereof, being operably linked to a promoter sequence active in plants and a terminator sequence active in plants; b) transforming a plant cell with the construct; and c) regenerating a transgenic plant from the transformed plant cell. Preferably, the 5'

untranslated region of the fructosyltransferase gene is modified such that the expression of the fruct syltransferase is not negatively affected. The DNA construct may also comprise a targeting sequence upstream from the fruct syltransferase gene for directing the fructosyltransferase to a specific plant tissue or plant cell compartment. The fructosyltransferases produced in the transgenic plants change the fructan pattern of the plants, thus leading to different plant performance due to altered sink-source relations and yield, increased tolerance for drought, cold or other stresses, higher dry matter content, better taste and storability, and improved nutritional value. The plants are also suitable for use as raw material for fructan production.

29 Claims, 9 Drawing figures

Exemplary Claim Number: 12

Number of Drawing Sheets: 9

----- KWIC -----

Abstract Text - ABTX:

The present invention relates to a method for obtaining transgenic plants showing a modified fructan pattern as compared to non-transformed plants, comprising the steps of: a) preparing a DNA construct comprising one or more fructosyltransferase genes, or modified versions thereof, being operably linked to a promoter sequence active in plants and a terminator sequence active in plants; b) transforming a plant cell with the construct; and c) regenerating a transgenic plant from the transformed plant cell. Preferably, the 5' untranslated region of the fructosyltransferase gene is modified such that the expression of the fructosyltransferase is not negatively affected. The DNA construct may also comprise a targeting sequence upstream from the fructosyltransferase gene for directing the fructosyltransferase to a specific plant tissue or plant cell compartment. The fructosyltransferases produced in the transgenic plants change the fructan pattern of the plants, thus leading to different plant performance due to altered sink-source relations and yield, increased tolerance for drought, cold or other stresses, higher dry matter content, better taste and storability, and improved nutritional value. The plants are also suitable for use as raw material for fructan production.

Brief Summary Text - BSTX:

The term "fructan pattern" refers to the distribution of the fructan in the different plant tissues and plant cell compartments such as cytosol, vacuole, apoplast etc. Some plants naturally do produce fructans whereas others do not. The fructan pattern of a plant species not producing fructans in the non-transformed plant may be changed by the introduction of a gene encoding a fructosyltransferase. The fructan distribution in a plant may also be changed by the redirection of the metabolic flow in plants or plant cells to certain plant or plant cell compartments.

Brief Summary Text - BSTX:

The fructosyltransferases of the present invention use sucrose as a substrate to synthesize high molecular weight fructans. Many micro-organisms contain fructosyltransferases which have the ability to produce fructans (often called levans) from sucrose (A. Fuchs 1959, Thesis, University of Leiden, Han 1989 Adv. Appl. Microbiol. 35, 171-194). These enzymes can transfer fructose moieties from sucrose to a fructan acceptor molecule to produce high molecular weight fructans. Since these fructan acceptor molecules are originally derived from sucrose they may still contain a terminal glucose molecule. The reaction mechanism is as follows (review see Han 1989 Adv. Appl. Microbiol. 35, 171-194): ##STR1##

Brief Summary Text - BSTX:

Two examples of fructosyltransferase producing bacteria are *Bacillus subtilis* (Steinmetz et al. 1985 Mol. Gen. Genet. 200, 220-228) and *Streptococcus mutans* (Shiroza, and Kuramitsu 1988 J. Bacteriol. 170, 810-816. In *B. subtilis* the *sacB* gene encodes the structural gene for levansucrase, a fructosyltransferase. This enzyme can convert sucrose into a fructose polymer with DP's which can easily exceed 10000. The linkage type found in this fructan produced by the levansucrase is mainly of the (2-6)-type with extensive (2-1)-branching.

Brief Summary Text - BSTX:

The present invention is not limited to naturally occurring fructosyltransferases but may equally well be performed by using modified versions thereof. Modifications may influence the activity of the fructosyltransferases in such a way that e.g. the degree of polymerization or the structure of the fructan produced is altered.

Detailed Description Text - DETX:

To create fructan-producing transgenic plants, genes encoding proteins capable of producing fructans were selected. One of these genes, levansucrase, encoded by the *sacB* gene of *Bacillus subtilis* (Steinmetz M. et al. Mol. Gen. Genet. 200:220-228(1985)), was used. This enzyme produces mainly branched fructans of the (2-6)-linkage type in the presence of sucrose.

Detailed Description Text - DETX:

Since sucrose in plant cells can accumulate in vacuoles, this is a preferred site for fructan production. To direct the levansucrase to the vacuole the targeting region of carboxypeptidase Y (Valls L. A. et al. Cell 48, 887-897 (1987)) was selected.

Detailed Description Text - DETX:

The fructans accumulating in these plants were further studied by isolation of larger quantities (Livingstone III D. A., Plant Physiol. 92, 767-769 (1990)). This fructan was analysed by sizing on a FPLC Superose 6HR 10/30 column (Pharmacia) and fructans were detected in the void volume indicating a degree of polymerisation of $>25,000$ fructose units. **Fructan produced** by *Bacillus subtilis* **levansucrase** similarly elutes in the void volume of the Superose column.

Detailed Description Text - DETX:

The purified fructans were also analysed by proton-NMR. Fructan isolated from transgenic plants showed no differences in peak pattern when compared with **fructan synthesised by levansucrase** from *Bacillus subtilis*.

Detailed Description Text - DETX:

To create **fructan-producing** plants, genes encoding proteins capable of producing fructans were selected. Another gene, **fructosyltransferase** encoded by the *ftf* gene of *Streptococcus mutans* (Shiroza T. and Kuramitsu H. K., J. Bacteriol. 170, 810-816 (1988)), was used. This enzyme produces mainly branched fructans of the (2-1)-linkage type in the presence of sucrose. Since sucrose in plant cells can accumulate in vacuoles, this is a preferred site for fructan accumulation. A gene encoding a protein capable of targeting itself to the vacuole was selected: carboxypeptidase Y (Valls L. A. et al., supra). From this gene the signal sequence and vacuolar targeting sequence were used as described in Example 1.

Detailed Description Text - DETX:

Screening of the transformants, using the TLC method as in Example 1, showed accumulation of fructan with the normal variation due to position effect. The fructans accumulating in these plants were further studied by isolation of larger quantities (Livingstone III D. A., supra). This fructan was analysed by sizing on a FPLC Superose, 6HR 10/30 column (Pharmacia) and fructans were detected in the void volume indicating a degree of polymerisation of $>25,000$ fructose units. **Fructan produced** by *Streptococcus mutans* **fructosyltransferase** similarly elutes in the void volume of the Superose column. Similarly partial fructan degradation by acid degradation shows a characteristic pattern of hydrolysis products. The purified plant and bacterial fructans show identical degradation patterns on TLC. Full acid hydrolysis followed by HPLC analysis on an Aminex HPX87C column at 85.degree. C. using water as eluent shows this fructan to be composed of fructose.

Detailed Description Text - DETX:

To create plants which produce fructans, genes encoding proteins capable of producing fructans were selected. Another gene, fructosyltransferase encoded by the ftf gene (Shiroza T. and Kuramitsu H. K., supra) and introduced in Example 2, was used. Since sucrose in plant cells is synthesized in the cytoplasm, the cytoplasm is a preferred site for fructan accumulation. Since nuclear-encoded proteins are made in the cytoplasm no targeting sequence is needed.

Claims Text - CLTX:

14. A DNA construct for the production of transgenic plants showing a distribution of fructan in their plant tissues and plant cell components differing from the distribution found in non-transformed plants, comprising one or more fructosyltransferase genes encoding fructosyltransferase protein that is enzymatically active when expressed in a plant cell wherein the 5' untranslated regions of the fructosyltransferase genes are deleted from the genes or otherwise inactivated, said one or more fructosyltransferase genes being operably linked to a plant specific promoter sequence and a plant specific terminator sequence.

Other Reference Publication - OREF:

Yamamoto, S., et al. "The Mode of Synthesis of Levan by Bacillus Subtillis Levansucrase", Agricultural and Biological Chemistry, vol. 49, No. 2, pp. 343-349 (Feb., 1985).

US-PAT-NO: 5985666

DOCUMENT-IDENTIFIER: US 5985666 A

TITLE: Forages

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------|-------|----------|---------|
| Loiselle; | Clive | IA | N/A | N/A |
| Fran.cedilla.ois J. | Johnston | IA | N/A | N/A |
| Nichols; Scott E. | | | | |

APPL-NO: 08/ 979514

DATE FILED: November 26, 1997

PARENT-CASE:

This application is a continuation-in-part of applications U.S. Ser. No. 08/478,704 now abandoned, U.S. Ser. No. 08/485,243, now U.S. Pat. No. 5,712,107; U.S. Ser. No. 08/482,711, now abandoned, all filed Jun. 7, 1995 .

US-CL-CURRENT: 435/419; 435/15 ; 435/193 ; 435/252.3 ; 435/278 ; 435/320.1 ; 435/410 ; 435/412 ; 536/123.12 ; 536/128 ; 536/23.2 ; 800/298 ; 800/320

ABSTRACT:

A transgenic plant cell is provided containing a DNA molecule encoding an enzyme selected from the group consisting of fructosyltransferase, glucosyltransferase B, mutants of glucosyltransferase B, glucosyltransferase C, glucosyltransferase D, mutants of glucosyltransferase D and functional fragments of each enzyme. A transgenic plant regenerated from the plant cell is also provided. A method of improving the ensilability and the nutritional value of plants is also provided comprising introducing into the cells of the plant an expression cassette comprising the above DNA molecule operably linked to a promoter functional in the cells of the plant to yield transformed plant cells, and regenerating a transformed plant from the transformed cells. The transformed plants also provide improved digestibility in ruminants.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Detailed Description Text - DETX:

Sprenger, N., et al., Purification, cloning, and functional expression of sucrose:fructan 6-**fructosyltransferase**, a key enzyme of **fructan synthesis** in barley, Proc. Natl. Acad. Sci. 92 (25), 11652-11656 (1995).

Detailed Description Text - DETX:

The production of the present transgenic plants is performed according to methods of transformation that are well known in the art. The glucans and fructans are synthesized by insertion of an expression cassette containing a structural gene which, when transcribed and translated, yields a glucosyltransferase or **fructosyltransferase** enzyme that **produces the desired glucan or fructan.**

US-PAT-NO: 5972631

DOCUMENT-IDENTIFIER: US 5972631 A

TITLE: Sucrose detection by enzyme-linked immunosorbant assay

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|------------------------------|----------------|-------|----------|---------|
| Borgford; Thor Jon | Burnaby | N/A | N/A | CA |
| Racher; Kathleen Iris | West Vancouver | N/A | N/A | CA |
| Braun; Curtis Archie John | Burnaby | N/A | N/A | CA |

APPL-NO: 08/ 962723

DATE FILED: November 3, 1997

US-CL-CURRENT: 435/7.9; 435/14 ; 435/15 ; 435/178 ; 435/18 ; 435/210 ; 435/7.1
; 435/7.32 ; 435/7.94 ; 436/518 ; 436/524

ABSTRACT:

A method is described for the rapid, sensitive and accurate determination of sucrose in biological fluids. A substrate is pre-coated with a glucose or fructose polymer and a transglycosidase enzyme. When the coated substrate is incubated with biological fluids containing concentrations of sucrose, the transglycosidase enzyme transfers monomers of glucose or fructose from the sucrose to the glucose or fructose polymer. The dimensions of the polymer are increased in proportion to the sucrose concentration of the samples. Newly formed polymer is subsequently quantitated in an immunoassay which employs either a combination of a carbohydrate-binding protein (which may be an antibody) and a conjugate of a secondary antibody and a marker enzyme, or a conjugate of a carbohydrate-binding protein and a marker enzyme. The assay is accurate at sucrose concentrations below 1 .mu.g/mL. No interference was observed at glucose concentrations as high as 25 mM. The sucrose detection assay is particularly useful in a non-invasive diagnostic test for gastric damage.

13 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

Detailed Description Text - DETX:

In this invention, sucrose is detected and measured by an indirect immunological method in which free sucrose is first converted to an antigenic polymer. Any of the three known transglycosidase enzymes, which accomplish the conversion of sucrose to a polymer comprised of one of its hexose constituents, glucose or fructose, may be used in this method. These enzymes are amylosucrase, dextransucrase, and levansucrase. The enzyme amylosucrase synthesizes a glycogen-like polymer (.alpha., 1-4 glycosidic linkages) from the glucose component of sucrose and releases fructose. Similarly, the enzyme dextransucrase synthesizes dextran (.beta., 1-6 glycosidic linkages) a polymer of glucose from sucrose and releases fructose. Levan sucrase differs from the former two enzymes in that it synthesizes levan (2-6, .alpha.-D fructan) a polymer of fructose while glucose is released. The preferred transglycosidase enzyme in the method of this invention is dextransucrase.

Detailed Description Text - DETX:

In a first preferred embodiment of this invention, the substrate is then treated with a carbohydrate binding protein which will bind to dextran, if the transglycosidase enzyme used in the method produces a dextran polymer, amylose if the transglycosidase enzyme used in the method produces an amylose polymer, or levan, if the enzyme produces a fructose polymer. An example of such carbohydrate binding proteins, which may be antibodies, is mouse anti-dextran antibody. In a preferred embodiment of the invention, wherein the glucose or fructose polymer is dextran and the transglycosidase enzyme is dextransucrase, the preferred antibody is mouse anti-dextran antibody. The antibody is typically added to the substrate in an aqueous solution, which may also contain buffers and surfactants. The preferred pH range for the buffered primer solution is from about 7.0 to about 8.0, most preferably from about 7.3 to about 7.5. Preferred buffer solutions include phosphate-buffered saline solution, or any other buffer solution with the appropriate pH range. The most preferred buffer solution is phosphate-buffered saline solution. The solution may also contain a surfactant. Suitable surfactants include, for example, Tween 20 and Triton X-100. Surfactants are typically present from about 0.1% to about 1% by weight of the solution, preferably from about 0.4% to about 0.6% by weight of the solution. The substrate and the antibody are incubated at a temperature in the range from about 21.degree. C. to about 42.degree. C., preferably from about 35.degree. C. to about 39.degree. C., and most preferably about 37.degree. C., for a period of about 15 minutes to about 120 minutes, preferably from about 30 minutes to about 45 minutes. Preferably, excess antibody is removed by washing the substrate. Typically, this is accomplished by means of multiple washings with distilled deionized water, or with a suitable buffer at a pH near neutrality.

US-PAT-NO: 5962278

DOCUMENT-IDENTIFIER: US 5962278 A

TITLE: Cellulose-producing bacteria

DATE-ISSUED: October 5, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------|-------|----------|---------|
| Tsuchida; Takayasu | Kawasaki | N/A | N/A | JP |
| Tonouchi; Naoto | Kawasaki | N/A | N/A | JP |
| Seto; Akira | Kawasaki | N/A | N/A | JP |
| Kojima; Yukiko | Kawasaki | N/A | N/A | JP |
| Matsuoka; Masanobu | Kawasaki | N/A | N/A | JP |
| Yoshinaga; Fumihiko | Kawasaki | N/A | N/A | JP |

APPL-NO: 08/ 973757

DATE FILED: February 3, 1998

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|----------------|
| JP | 8/123951 | April 23, 1996 |
| JP | 8/149763 | May 22, 1996 |
| JP | 8/174393 | June 14, 1996 |

PCT-DATA:

APPL-NO: PCT/JP97/00514
DATE-FILED: February 24, 1997
PUB-NO: WO97/40135
PUB-DATE: Oct 30, 1997
371-DATE: Feb 3, 1998
102(E)-DATE: Feb 3, 1998

US-CL-CURRENT: 435/101; 435/252.1 ; 435/823

ABSTRACT:

This invention relates to a microorganism that is capable of producing a cellulosic product (referred to hereinafter as a "cellulose-producing bacterium") and belongs to a novel subspecies which is substantially negative or very slightly positive in oxidation of acetates and lactates. This invention also relates to novel saccharide analog-resistant strains, amino acid analog-resistant strains and levan sucrase-defective strains.

Further, this invention relates to a method for the production of cellulosic material (bacterial cellulose: "BC"), which comprises culturing these novel bacteria and to bacterial cellulose which may be thus obtained.

A larger amount of bacterial cellulose may be produced by culturing *Acetobacter xylinum* subsp. *nonacetoxidans*, the present resistant strains and the levan sucrase-defective strains, which have been derived and bred from the cellulose-producing bacteria, than by culturing the BPR 2001 strain in the medium containing especially sucrose or glucose as carbon sources.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Mutants include a levan sucrase-defective mutant in which the production of levan is suppressed.

Brief Summary Text - BSTX:

Levan sucrase (EC2.4.1.10) is known to decompose and metabolize sucrose. This enzyme has two activities, (1) hydrolysis activity of sucrose into glucose and fructose, and (2) transfructosylation activity to produce glucose and levan from sucrose. The latter activity is not preferred in terms of BC production since it will produce levan as a by-product. It will be very advantageous in the production and purification of BC, if the accumulation of levan is suppressed. The present inventors have already disclosed in the Japanese Patent Application Hei 7 (1995)-252021 that by deriving the levan sucrase-defective strain and incorporating the gene of an extracellular invertase or a levan sucrase with a reduced levan productivity into said strain, the accumulation of levan will be reduced and the cellulose may be efficiently produced.

Detailed Description Text - DETX:

The 757-3-5-11 strain was subjected to the same mutagenesis treatment as above, and inoculated on a plate containing the minimum medium shown in Table 10 using sucrose instead of glucose. The small non-mucoidal colonies producing no levan were selected as candidate strains. Further, the two strains (LD-1 and LD-2) producing cellulose well in the Glc-CSL medium (Table 11) were selected from the above candidates. Table 24 shows the production of cellulose and polysaccharide as by-product by these two strains in CSL-Glc and CSL-Suc media (Table 5). No activity of levan sucrase was observed in these two strains by the usual method (H. Yanase et al., Biosci. Biotech. Biochem., Vol. 55 pp. 1383-1390, 1991), confirming that these two strains are levan sucrase-defective strains.

Detailed Description Text - DETX:

The levan sucrase-defective strain, LD-2 was transformed with the plasmid pSAZE3S containing the exocellular invertase gene and its secretion-accelerating gene of *Zymomonas mobilis* strain disclosed in the Japanese Patent Application Hei 7 (1995)-252021, or the plasmid pSARH containing the gene of *Bacillus subtilis* encoding a levan sucrase with its reduced activity for levan formation. The production of cellulose by these transformants in the CSL-Suc medium (Table 5) is shown in Table 25. The introduction of the above genes into the levan sucrase-defective strain has remarkably reduced the amount of levan as by-product in the production of cellulose from sucrose.

US-PAT-NO: 5952314

DOCUMENT-IDENTIFIER: US 5952314 A

TITLE: Nutritional product for a person having ulcerative colitis

DATE-ISSUED: September 14, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-------------|-------|-----------|---------|
| DeMichele; Stephen | Dublin | OH | 43017 | N/A |
| Joseph | Powell | OH | 43081 | N/A |
| Garleb; Keith Allen | Gahanna | OH | 43230 | N/A |
| McEwen; John William | Westerville | OH | 43081-360 | N/A |
| Fuller; Martha Kay | | 2 | | |

APPL-NO: 09/ 083736

DATE FILED: May 22, 1998

PARENT-CASE:

This application is a continuation-in-part of application Ser. No. 08/221,349, now U.S. Pat. No. 5,780,451, filed on Apr. 1, 1994.

US-CL-CURRENT: 514/54; 426/567 ; 426/658 ; 514/168 ; 514/188 ; 514/552 ; 514/566 ; 514/725 ; 514/810 ; 514/812 ; 514/813 ; 514/861

ABSTRACT:

An enteral nutritional product for a person having ulcerative colitis contains in combination (a) an oil blend which contains eicosapentaenoic acid (20:5n3) and/or docosahexaenoic acid (22:6n3), and (b) a source of indigestible carbohydrate which is metabolized to short chain fatty acids by microorganisms present in the human colon. Preferably the nutritional product also contains one or more nutrients which act as antioxidants.

16 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

In the first experiment the objective was to determine short chain fatty acid

production from a variety of indigestible oligosaccharides during fermentation with mixed human fecal microbiota. Several indigestible oligosaccharides were tested including FOS, Raftilose.RTM. and XOS. FOS is a fructooligosaccharide produced on a commercial scale by fermenting granulated sucrose in water with a pure strain of *Aspergillus niger*. The organism produces a fructosyltransferase enzyme which links additional fructose units onto the fructose end of sucrose molecules to produce 1-kestose (GF.sub.2), nystose (GF.sub.3) and 1.sup.F-.beta.-fructo-furanosylnystose (GF.sub.4). Raftilose.RTM. is a fructooligosaccharide produced via enzymatic hydrolysis of inulin, which is marketed by Rhone-Poulenc (Raffinerie Tirlemontoise SA). The hydrolysis results in a wide array of oligosaccharides such as GF.sub.2, GF.sub.3 and GF.sub.4 as well as oligosaccharides containing just fructose (F.sub.3, F.sub.4, F.sub.5, etc.). XOS is a xylooligosaccharide produced via enzymatic hydrolysis of xylan. The primary ingredients of XOS are xylobiose, xylotriose and xylotetrose.

US-PAT-NO: 5910438

DOCUMENT-IDENTIFIER: US 5910438 A

TITLE: Cloning and/or sequencing vector

DATE-ISSUED: June 8, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|----------|-------|----------|---------|
| Bernard; Philippe | Brussels | N/A | N/A | BE |
| Gabant; Philippe | Brussels | N/A | N/A | BE |

APPL-NO: 08/ 379614

DATE FILED: July 20, 1995

PARENT-CASE:

This application is a 371 of PCT/BE93/00051 filed Aug. 2, 1993.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| BE | 9200696 | July 31, 1992 |

PCT-DATA:

APPL-NO: PCT/BE93/00051
DATE-FILED: August 2, 1993
PUB-NO: WO94/03616
PUB-DATE: Feb 17, 1994
371-DATE: Jul 20, 1995
102(E)-DATE: Jul 20, 1995

US-CL-CURRENT: 435/252.3; 435/252.33 ; 435/320.1

ABSTRACT:

A cloning and/or sequencing vector (1) comprises a nucleotide promoter sequence (3) and a nucleotide sequence (4) coding for a fusion protein active as a poison, which nucleotide sequence (4) is obtained by fusion of a coding nucleotide sequence (5) containing several unique cloning sites with a nucleotide sequence (6) coding for a poison protein, wherein the nucleotide sequences (3) and (4) are incorporated in an autonomous replication vector (2).

14 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

Brief Summary Text - BSTX:

Since the gene sac B encodes levan sucrase, which catalyzes the hydrolysis of sucrose into products which are toxic for E. coli, direct selection of the mutants which incorporate a recombinant plasmid is effected on a culture medium containing sucrose. Since the levan sucrase is toxic, even in the absence of sucrose, it is essential, consequently, to repress its synthesis if one wishes to obtain a large number of plasmid copies in the bacterial cytoplasm.

US-PAT-NO: 5908975

DOCUMENT-IDENTIFIER: US 5908975 A

TITLE: Accumulation of fructans in plants by targeted expression of bacterial levansucrase

DATE-ISSUED: June 1, 1999

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|--------------|-------|----------|---------|
| Caimi; Perry Gerard | Landenberg | PA | N/A | N/A |
| Hershey; Howard Paul | West Chester | PA | N/A | N/A |
| Kerr; Phillip S. | Urbandale | IA | N/A | N/A |

APPL-NO: 08/ 640732

DATE FILED: May 6, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This is the national stage of international application PCT/US94/12778, filed Nov. 7, 1994, which is a continuation-in-part of U.S. patent application Ser. No. 08/149,689, filed Nov. 9, 1993 now abandoned.

PCT-DATA:

APPL-NO: PCT/US94/12778
DATE-FILED: November 7, 1994
PUB-NO: WO95/13389
PUB-DATE: May 18, 1995
371-DATE: May 6, 1996
102(E)-DATE: May 6, 1996

US-CL-CURRENT: 800/298; 435/320.1 ; 435/419 ; 435/468 ; 536/23.2 ; 536/23.7 ; 800/317.2 ; 800/317.3 ; 800/320.1

ABSTRACT:

This invention concerns methods for synthesis and accumulation of fructose polymers in seed, tubers or leaves of transgenic plants by selective expression of a bacterial **fructosyltransferase** gene. Selective expression includes coordination of timing, tissue specific expression and especially subcellular location. Successful transformants utilize sucrose to **synthesize and accumulate fructan** in the vacuole of the cell, in established crops, without loss of co-products or concern for yield loss due to degradation during maturation, harvest or storage of the plant. Enhanced **fructan production** will benefit the fructose sweetener industry and add value to grain used for feed.

11 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Abstract Text - ABTX:

This invention concerns methods for synthesis and accumulation of fructose polymers in seed, tubers or leaves of transgenic plants by selective expression of a bacterial fructosyltransferase gene. Selective expression includes coordination of timing, tissue specific expression and especially subcellular location. Successful transformants utilize sucrose to synthesize and accumulate fructan in the vacuole of the cell, in established crops, without loss of co-products or concern for yield loss due to degradation during maturation, harvest or storage of the plant. Enhanced fructan production will benefit the fructose sweetener industry and add value to grain used for feed.

Brief Summary Text - BSTX:

Fructan accumulation in the field is extremely sensitive to environmental change. Exposure to drought or frost dramatically alters the quality of the fructan accumulated (Praznik and Beck, Agr. Biol. Chem., 51:1593-1599 (1987)). Traditional breeding programs could in theory, result in varieties with reduced quality losses due to environmental change. However, programs of this type, normally very time consuming, are not in place at this time and would likely be implemented only when the fructan industry proves to be viable. Genetic engineering of fructan containing crops could also eliminate these barriers. Overexpression of a fructan biosynthetic gene or genes, may lead to increased yield, synthesis of larger molecular weight fructans or reduced quality losses due to frost or drought. This approach could also potentially eliminate the need for specialized storage conditions. Success of such a genetic program would rely heavily on a detailed understanding of the biochemistry of fructan synthesis, the kinetics of the biosynthetic proteins and ultimately, understanding the regulation of the genes involved in fructan synthesis. At present, this knowledge is lacking. The current model for all fructan accumulating plants, proposed in 1968 (Eddleman and Jefford, New Phytol. 67:517-531 (1968)), suggests that polymer synthesis and storage is achieved by the sequential action of two separate proteins. The model, which has been slightly altered, (Wagner et al., Zeitschrift für Pflanzenphysiologie 112:359-372, (1983); Frehner et al. 1984, New Phytol. 116:197-208) has yet to resolve a key issue regarding reversible fructosyltransferase (FTF) activity and is once again under critical review (Housley et al., New Phytol. 119:491-97, (1991); Cairns, A. J., New Phytol. 120:463-73 (1992)). Enzymes involved in the biosynthesis pathway have not been purified to homogeneity. Therefore, attempts to fully understand fructan metabolism and then to alter regulation of synthesis, control loss due to degradation and increase the molecular weight of accumulated fructans in a transgenic plant using a cloned plant gene or genes may be several years away.

Brief Summary Text - BSTX:

This invention concerns a recombinant DNA construct comprising a tissue specific promoter, operably linked to a vacuole targeting sequence, operably linked to a coding sequence for a levansucrase gene such that said construct is capable of transforming a plant cell selected from the group consisting of corn, potato, and tobacco to obtain production of fructan in the vacuole of said plant cell without deleterious effect on said plant cell. The invention also concerns a plant selected from the group consisting of corn, potato, and tobacco transformed with said construct, such that said plant produces fructan which accumulates in the vacuole of the cells of the plant. The invention further concerns a method of producing fructose comprising growing the said described plant, harvesting said plant, and extracting said fructan from the harvested plant.

Claims Text - CLTX:

1. A recombinant DNA construct comprising a tissue specific promoter, operably linked to a vacuole targeting sequence, operably linked to a coding sequence for a bacterial levansucrase gene wherein transformation of a plant cell selected from the group consisting of corn, potato, and tobacco with said construct results in production of fructan in the vacuole of said plant cell.

Claims Text - CLTX:

8. A method of increasing fructan levels in plants, comprising transforming a plant cell that has a higher than native sucrose level with a recombinant DNA construct comprising a tissue specific promoter, operably linked to a vacuole targeting sequence, operably linked to a coding sequence of a bacterial levansucrase gene, and growing a fertile, sexually mature, transformed plant from the transformed plant cell, wherein the transformed plant synthesizes and accumulates fructan.

US-PAT-NO: 5792923

DOCUMENT-IDENTIFIER: US 5792923 A

TITLE: DNA sequences which lead to the formation of levans plasmids containing these sequences as well as a process for preparing transgenic plants

DATE-ISSUED: August 11, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|--------------------|-------|----------|---------|
| Rober; Manuela | Berlin | N/A | N/A | DE |
| Geier; Gebhard | Heidelberg/Dossenh | N/A | N/A | DE |
| Geider; Klaus | eim | N/A | N/A | DE |
| Willmitzer; Lothar | Sandhausen | N/A | N/A | DE |
| | Berlin | | | |

APPL-NO: 08/ 381936

DATE FILED: February 9, 1995

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|-------------|-----------------|
| DE | 42 27 061.8 | August 12, 1992 |

PCT-DATA:

APPL-NO: PCT/EP93/02110
DATE-FILED: August 9, 1993
PUB-NO: WO94/04692
PUB-DATE: Mar 3, 1994
371-DATE: Feb 9, 1995
102(E)-DATE: Feb 9, 1995

US-CL-CURRENT: 800/284; 435/101 ; 435/103 ; 435/200 ; 435/210 ; 435/211 ; 435/320.1 ; 435/419 ; 435/69.1 ; 536/23.7 ; 800/298 ; 800/317.2 ; 800/317.3

ABSTRACT:

There are described DNA sequences, which lead to the formation of levans, plasmids containing these DNA sequences, as well as a process using these plasmids for preparing transgenic plants with levan accumulation.

32 Claims, 5 Drawing figures

Exemplary Claim Number: 1,4

Number of Drawing Sheets: 5

----- KWIC -----

Brief Summary Text - BSTX:

Until now, genes for levan sucrase from *Bacillus amyloliquefaciens* (Tang et al. (1990) Gene 96, 89-93) and *Bacillus subtilis* (Steinmetz et al. (1985) Mol. Gen. Genetics 200, 220-228), have been described, and demonstrate relatively high homology with each other and both of which catalyse the synthesis of fructans of the levan type. Further a fructosyl transferase from *Streptococcus mutans* (Shiroza et al. (1988) J. Bacteriology 170, 810-816) has been described. This shows little homology to either levan sucraes from *Bacillus* spp. The fructan formed in *Streptococcus mutans* is of the inulin type.

Brief Summary Text - BSTX:

Since sucrose represents the substrate for the levan sucrase, the production of polyfructans is especially advantageous in those organs which store large amounts of sucrose. Such organs are for example, the roots of sugar beet or the stems of sugar cane. It is especially useful in genetically modified potatoes, which store sucrose in their tubers, through the blocking of starch biosynthesis.

US-PAT-NO: 5792630

DOCUMENT-IDENTIFIER: US 5792630 A

TITLE: Cellulose-producing microorganism transformed with a gene for an enzyme involved in sucrose metabolism

DATE-ISSUED: August 11, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------|-------|----------|---------|
| Tonouchi; Naoto | Kawasaki | N/A | N/A | JP |
| Tsuchida; Takayasu | Kawasaki | N/A | N/A | JP |
| Yoshinaga; Fumihito | Kawasaki | N/A | N/A | JP |
| Horinouchi; Sueharu | Tokyo | N/A | N/A | JP |
| Beppu; Teruhiko | Tokyo | N/A | N/A | JP |
| Yanase; Hideshi | Tottori | N/A | N/A | JP |
| Hayashi; Takahisa | Uji | N/A | N/A | JP |

APPL-NO: 08/ 578536

DATE FILED: March 29, 1996

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-----------------|
| JP | 6-127995 | May 19, 1994 |
| JP | 6-222751 | August 25, 1994 |

PCT-DATA:

APPL-NO: PCT/JP95/00961
DATE-FILED: May 19, 1995
PUB-NO: WO95/32279
PUB-DATE: Nov 30, 1995
371-DATE: Mar 29, 1996
102(E)-DATE: Mar 29, 1996

US-CL-CURRENT: 435/101; 435/193 ; 435/194 ; 435/252.1 ; 435/252.33 ; 435/320.1 ; 435/69.1 ; 435/823 ; 530/350 ; 536/23.2

ABSTRACT:

A method for the production of a cellulosic product, which comprises:

culturing a cellulose-producing microorganism transformed with a gene for an enzyme involved in sucrose metabolism in a medium containing sucrose, allowing the cellulosic product to be produced and accumulated in the medium, and collecting the cellulosic product. By the present method, the cellulosic product can be produced efficiently and economically.

26 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Brief Summary Text - BSTX:

Levan sucrase (EC2.4.1.10) is also known to decompose and metabolize sucrose. This enzyme has two activities, (1) hydrolysis activity of sucrose into glucose and fructose, and (2) transfructosylation activity to produce glucose and levan from sucrose. The latter activity is not preferred in terms of BC production since it will produce levan as by-product.

Detailed Description Text - DETX:

Comparison of amino acid sequences between the exocellular levan sucrase gene and exocellular invertase gene of *Zymomonas mobilis* IFO 13756 (Z6) strain revealed that His.sup.297 at a specificity-determining region in downstreams of the levan sucrase corresponded to Asp in the invertase. An expression vector H297D comprising a variant-type enzyme gene was created from the expression vector for the levan sucrase gene, pUZE2d (Abstracts of Annual Meeting of the Society for Fermentation and Bioengineering, Japan, p.20, 1992) by site-directed mutagenesis according to the method of Kunkel, et al., Methods in Enzymology, 154, 367, (1987), which substituted Asp for His.sup.297 of the levan sucrase by using a synthesized oligonucleotide having the sequence:

US-PAT-NO: 5780451

DOCUMENT-IDENTIFIER: US 5780451 A

TITLE: Nutritional product for a person having ulcerative colitis

DATE-ISSUED: July 14, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-------------|-------|----------|---------|
| DeMichele; Stephen | Dublin | OH | N/A | N/A |
| Joseph | Powell | OH | N/A | N/A |
| Garleb; Keith Allen | Gahanna | OH | N/A | N/A |
| McEwen; John William | Westerville | OH | N/A | N/A |
| Fuller; Martha Kay | | | | |

APPL-NO: 08/ 221349

DATE FILED: April 1, 1994

US-CL-CURRENT: 514/54; 426/567 ; 426/658 ; 514/168 ; 514/188 ; 514/552
; 514/566 ; 514/725 ; 514/810 ; 514/812 ; 514/813 ; 514/861

ABSTRACT:

An enteral nutritional product for a person having ulcerative colitis contains in combination (a) an oil blend which contains eicosapentaenoic acid (20:5n3) and/or docosahexaenoic acid (22:6n3), and (b) a source of indigestible carbohydrate which is metabolized to short chain fatty acids by microorganisms present in the human colon. Preferably the nutritional product also contains one or more nutrients which act as antioxidants.

18 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

In the first experiment the objective was to determine short chain fatty acid production from a variety of indigestible oligosaccharides during fermentation with mixed human fecal microbiota. Several indigestible oligosaccharides were tested including FOS, Raftilose.RTM. and XOS. FOS is a fructooligosaccharide produced on a commercial scale by fermenting granulated sucrose in water with a pure strain of *Aspergillus niger*. The organism produces a fructosyltransferase

enzyme which links additional fructose units onto the fructose end of sucrose molecules to produce 1-kestose (GF.sub.2), nystose (GF.sub.3) and 1.sup.F-B-fructo-furanosylnystose (GF.sub.4). Raftilose.RTM. is a fructooligosaccharide **produced via enzymatic hydrolysis of inulin**, which is marketed by Rhone-Poulenc (Raffinerie Tirlemontoise SA). The hydrolysis results in a wide array of oligosaccharides such as GF.sub.2, GF.sub.3 and GF.sub.4 as well as oligosaccharides containing just fructose (F.sub.3, F.sub.4, F.sub.5, etc.). XOS is a xylooligosaccharide produced via enzymatic hydrolysis of xylan. The primary ingredients of XOS are xylobiose, xylotriose and xylotetrose.

US-PAT-NO: 5731173

DOCUMENT-IDENTIFIER: US 5731173 A

TITLE: Fructosyltransferase enzyme, method for its production and DNA encoding the enzyme

DATE-ISSUED: March 24, 1998

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------------|--------|-------|----------|---------|
| Sosa; Juan Gabriel | Havana | N/A | N/A | CU |
| Arrieta | Havana | N/A | N/A | CU |
| Garcia; Lazaro | Havana | N/A | N/A | CU |
| Hernandez | Havana | N/A | N/A | CU |
| Gonzalez; Alberto Coego | | | | |
| Sosa; Guillermo | | | | |
| Selman-Housein | | | | |

APPL-NO: 08/ 814196

DATE FILED: March 10, 1997

PARENT-CASE:

This application is a division of U.S. Ser. No. 08/362,232 filed on Dec. 22, 1994 now U.S. Pat. No. 5,644,667.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|-------------------|
| CU | 125/93 | December 23, 1993 |

US-CL-CURRENT: 435/97; 435/193 ; 435/823

ABSTRACT:

Extracellular fructosyltransferase of *Acetobacter diazotrophicus* was isolated and purified and its enzymatic properties were established. Cloning, sequencing and genetic manipulation of the fructosyltransferase gene so as to produce high levels of the enzyme in recombinant prokaryotic and eukaryotic cells. Both natural and recombinant fructosyltransferase of *Acetobacter diazotrophicus* produce fructose-containing oligosaccharides and fructans. The enzyme yields in particular high levels of fructooligosaccharides from sucrose, such as kestose and kestotetraose which can be used as natural low-calorie sweeteners.

6 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Acetobacter diazotrophicus secretes a constitutive fructosyltransferase with levansucrase activity. The enzyme has utility for the production of fructose-containing oligosaccharides and levan. Fructose polymers have two characteristic properties, their nondigestibility and selective utilization by beneficial intestinal bacteria, which make them useful as low-calorie dietary fiber for relief of constipation, improvement of blood lipid composition, cholesterol reduction, and suppression of intestinal putrefactive substances. During the course of sucrose transformation the Acetobacter diazotrophicus fructosyltransferase accumulates a high yield of fructooligosaccharides, particularly kestose and kestotetraose which can serve as natural low-calorie sweeteners. Levan can also be used as a source of fructose, a blood plasma volume extender, an emulsifier, an encapsulating agent, etc.

Brief Summary Text - BSTX:

The fructosyltransferase of Acetobacter diazotrophicus is a levansucrase which yields a high level of oligofructans of low polymerization degree. During the course of sucrose transformation 55% of fructose transferred by the enzyme is accumulated as kestotriose and kestotetraose. These fructooligosaccharides are high quality sweeteners with many applications in the food industry. The enzyme can be efficiently applied, therefore, for producing kestose and kestotetraose from sucrose, and it is also useful in the production of high weight levan.

Brief Summary Text - BSTX:

The invention also comprises the nucleotide sequence of the fructosyltransferase gene of Acetobacter diazotrophicus isolated from a genomic library by complementing EMS-treated mutants of A. diazotrophicus unable to produce levan.

US-PAT-NO: 5641667

DOCUMENT-IDENTIFIER: US 5641667 A

TITLE: Fructosyltransferase enzyme, method for its production and DNA encoding the enzyme

DATE-ISSUED: June 24, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------------|--------|-------|----------|---------|
| Sosa; Juan Gabriel | Vedado | N/A | N/A | CU |
| Arrieta | Playa | N/A | N/A | CU |
| Hernandez Garcia; | Cerro | N/A | N/A | CU |
| Lazaro | Playa | N/A | N/A | CU |
| Gonzalez; Alberto Coego | | | | |
| Sosa; Guillermo | | | | |
| Selman-Housein | | | | |

APPL-NO: 08/ 362232

DATE FILED: December 22, 1994

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|-------------------|
| CU | 125/93 | December 23, 1993 |

US-CL-CURRENT: 435/193; 435/252.3 ; 435/252.33 ; 435/254.11 ; 435/254.23 ; 435/320.1 ; 536/23.2

ABSTRACT:

Extracellular fructosyltransferase of *Acetobacter diazotrophicus* was isolated and purified and its enzymatic properties were established. Cloning, sequencing and genetic manipulation of the fructosyltransferase gene so as to produce high levels of the enzyme in recombinant prokaryotic and eukaryotic cells. Both natural and recombinant fructosyltransferase of *Acetobacter diazotrophicus* produce fructose-containing oligosaccharides and fructans. The enzyme yields in particular high levels of fructooligosaccharides from sucrose, such as kestose and kestotetraose which can be used as natural low-calorie sweeteners.

20 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Acetobacter diazotrophicus secretes a constitutive fructosyltransferase with levansucrase activity. The enzyme has utility for the production of fructose-containing oligosaccharides and levan. Fructose polymers have two characteristic properties, their nondigestibility and selective utilization by beneficial intestinal bacteria, which make them useful as low-calorie dietary fiber for relief of constipation, improvement of blood lipid composition, cholesterol reduction, and suppression of intestinal putrefactive substances. During the course of sucrose transformation the Acetobacter diazotrophicus fructosyltransferase accumulates a high yield of fructooligosaccharides, particularly kestose and kestotetraose which can serve as natural low-calorie sweeteners. Levan can also be used as a source of fructose, a blood plasma volume extender, an emulsifier, an encapsulating agent, etc.

Brief Summary Text - BSTX:

The fructosyltransferase of Acetobacter diazotrophicus is a levansucrase which yields a high level of oligofructans of low polymerization degree. During the course of sucrose transformation 55% of fructose transferred by the enzyme is accumulated as kestotriose and kestotetraose. These fructooligosaccharides are high quality sweeteners with many applications in the food industry. The enzyme can be efficiently applied, therefore, for producing kestose and kestotetraose from sucrose, and it is also useful in the production of high weight levan.

Brief Summary Text - BSTX:

The invention also comprises the nucleotide sequence of the fructosyltransferase gene of Acetobacter diazotrophicus isolated from a genomic library by complementing EMS-treated mutants of A. diazotrophicus unable to produce levan.

US-PAT-NO: 5633154

DOCUMENT-IDENTIFIER: US 5633154 A

TITLE: Method for location of insertion elements

DATE-ISSUED: May 27, 1997

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-----------|-------|----------|---------|
| Schaefer, Andreas | Bielefeld | N/A | N/A | DE |
| Seep-Feldhaus; | Bielefeld | N/A | N/A | DE |
| Anna-Hildegard | Bielefeld | N/A | N/A | DE |
| Jaeger, Wolfgang | Bielefeld | N/A | N/A | DE |
| Kalinowski, Joern | Bielefeld | N/A | N/A | DE |
| Wohlleben, Wolfgang | Bielefeld | N/A | N/A | DE |
| Puehler, Alfred | | | | |

APPL-NO: 08/ 336069

DATE FILED: November 4, 1994

PARENT-CASE:

This is a divisional of application Ser. No. 08/033,320 filed on Mar. 18, 1993, U.S. Pat. No. 5,380,657.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|-------------|----------------|
| DE | 42 08 785.6 | March 19, 1992 |

US-CL-CURRENT: 435/473; 435/477 ; 435/487 ; 435/843

ABSTRACT:

A method of locating insertion elements (IS elements) or transposons in coryneform bacteria, a positive selection system suitable for the above, the IS elements found in this manner and their use, is disclosed. The method involves:

(1) The construction of a non-self-transferrable vector mobilizable from an E. coli mobilizer strain which vector is composed of

(a) A DNA segment containing a replicon functional in E. coli,

(b) A second DNA segment containing the DNA fragment coding for the mobilization function (Mob site containing the oriT),

(c) A third DNA segment which recombines homologously in Gram-positive bacteria and/or contains a replicon functional in coryneform bacteria,

- (d) A DNA segment from *Bacillus subtilis* containing the *sacB* gene,
- (2) Transfer of this vector by means of conjugative transfer into the coryneform recipient strains,
- (3) Cultivation of the transconjugants containing the vector in an about 10% sucrose-containing nutrient medium,
- (4) Lysis of the sucrose-resistant clones, cleaving of the plasmids with restriction endonucleases and analysis of the fragments.

2 Claims, 8 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

The *sacB* gene from *Bacillus subtilis* is used for the isolation of insertion sequences (IS elements) or transposons in accordance with the invention Gay et al., J. Bacteriol. (1983), volume 153, pages 1424-1431). The gene codes for the exoenzyme levam sucrase, which catalyzes the reactions of saccharose hydrolysis and levan synthesis (Dedonder et al., Methods in Enzymol. (1966), volume 8, pages 500-505). The expression of *sacB* in *E. coli* results in the transport of the enzyme into the periplasma (Steinmetz et al., Mol. Gen. Genet. (1983), volume 191, pages 138-144) and is lethal for *E. coli* and other Gram-negative bacteria placed on media containing over 5% sucrose (Gay et al., J. Bacteriol., volume 194, pages 918-921).

Detailed Description Text - DETX:

The *sacB* gene from *Bacillus subtilis* is used for the isolation of insertion sequences (IS elements) or transposons Gay et al., J. Bacteriol. (1983), volume 153, pages 1424-1431). The gene codes for the exoenzyme levam sucrase, which catalyses the reactions of saccharose hydrolysis and levan synthesis (Dedonder et al., Methods in Enzymol. (1966), volume 8, pages 500-505). The expression of *sacB* in *E. coli* results in the transport of the enzyme into the periplasma (Steinmetz et al., Mol. Gen. Genet. (1983), volume 191, pages 138-144) and is lethal for *E. coli* and other Gram-negative bacteria on media with over 5% sucrose (Gay et al, J. Bacteriol., volume 164, pages 913-921).

US-PAT-NO: 5547863

DOCUMENT-IDENTIFIER: US 5547863 A

TITLE: Production of fructan (levan) polyfructose polymers using bacillus polymyxa

DATE-ISSUED: August 20, 1996

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-------------|-------|----------|---------|
| Han; Youn W. | New Orleans | LA | N/A | N/A |
| Clarke; Margaret A. | New Orleans | LA | N/A | N/A |

APPL-NO: 07/ 393604

DATE FILED: August 14, 1989

US-CL-CURRENT: 435/101; 435/838 ; 536/114 ; 536/4.1

ABSTRACT:

Soil isolates, identified as strains of Bacillus polymyxa, NRRL B-18475 and NRRL B-18476, produce large quantities of a pure and uniform extracellular polysaccharide fructan (levan), in a sucrose medium. The levan consists entirely of fructose and the residues linked by .beta., 2-6 fructofuranoside linkage.

5 Claims, 0 Drawing figures

Exemplary Claim Number: 2

----- KWIC -----

Detailed Description Text - DETX:

The **levan produced** by the isolate B. polymyxa consisted of about 98% fructose as revealed by HPLC of the acid hydrolysate. The product was readily soluble in water and insoluble in 75% alcohol at room temperature. In contrast to low solubility of inulin (.beta.,2-1 linkage) the high solubility of the product may be a characteristic of .beta.,2-6 linked levans. The product was very susceptible to hydrolysis in boiling 0.5% oxalic acid. Since the initial molecule in levan formation is sucrose, terminal glucose groups are expected to be present in levan chains. However, because of the small portion of terminal groups in their high molecular weight levan, essentially no glucose was observed on hydrolysis or on methylation analysis. On gel permeation chromatography, a 5% aqueous solution of crude levan, after dialysis through a membrane with 12,000 daltons cut-off, gave a single, sharp clean peak about

2.times.10.sup.6 daltons on Sephacryl S-500. While the polymer of the present invention may have a range of molecular weights of from about 1.5.times.10.sup.6 to about 2.5'10.sup.6 daltons, the aforementioned peak is sharper (narrower molecular weight range) than those of the commercially available dextrans used as GPC standards. The uniformity of the product is perhaps due to the result of a long fermentation period (up to 10 days) and lack of levan-hydrolyzing activity in the levan-sucrase of the organism. Levan hydrolyzing activity is commonly found in other levan producers. The compound is stable in aqueous solution at pH 4.5 for up to 36 hr when monitored by HPLC analysis. The degree of optical rotation was determined using a polarimeter (Type AA-10, Optoelectronic Design Engineers, LTD., England) with a sodium lamp and a 100 mm sample tube. The amount of fructose in the levan hydrolysate was determined by comparing the degree of optical. rotation produced by the sample and the standard fructose solution. While various samples of the polymer of the present invention may exhibit negative optical rotation [.varies.]D.sup.24 of from about -40 to about -46 depending upon the degree of branching, at optimum level of production the optical rotation [.varies.]D.sup.24 will be -42.0. It is non-hygroscopic, which is unusual in view of its high solubility; this advantage facilitates ease of handling and storing. Lyophilized sheets of levan have been maintained under atmospheric condition for up to six months.

US-PAT-NO: 5527784

DOCUMENT-IDENTIFIER: US 5527784 A

TITLE: Antihyperlipidemic and antiobesity agent comprising levan or hydrolysis products thereof obtained from Streptococcus salivarius

DATE-ISSUED: June 18, 1996

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|---------------|-------|----------|---------|
| Ishihara; Kazuoki | Hachioji-shi, | N/A | N/A | JP |
| | Tokyo | | | |

APPL-NO: 08/ 357770

DATE FILED: December 16, 1994

PARENT-CASE:

This is a division of application Ser. No. 08/081,604, filed Jun. 8, 1993 now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|---------------|
| JP | 4-194472 | June 10, 1992 |

US-CL-CURRENT: 514/54; 435/885 ; 536/119

ABSTRACT:

The present invention relates to an antihyperlipidemic and antiobesity agent which has levan and/or a partial hydrolysate of levan as its active ingredient in order to provide an agent which can suppress increases in lipids in the blood serum and increases in body fat even when high-calorie foods such as carbohydrates are eaten; and at the same time, it is easy to take in effective quantities and there are no adverse side effects or toxicity.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

The antihyperlipidemic and antiobesity agent in the present invention has levan and/or a partial hydrolysate of levan as its active ingredient. The levan is

pr. duced by bacteria such as the Streptococcus salivarius and Bacillus subtilis, etc. It is also pr. duced by the enzymes (levan sucrase) that are pr. duced by these bacteria. The partial hydrolysate of levan is prepared by acid hydrolysis of levan under mild conditions.

US-PAT-NO: 5444054

DOCUMENT-IDENTIFIER: US 5444054 A

TITLE: Method of treating ulcerative colitis

DATE-ISSUED: August 22, 1995

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|--------|-------|----------|---------|
| Garleb; Keith A. | Powell | OH | N/A | N/A |
| Demichele; Stephen J. | Dublin | OH | N/A | N/A |

APPL-NO: 08/ 221440

DATE FILED: April 1, 1994

US-CL-CURRENT: 514/54; 426/72 ; 514/867 ; 514/925

ABSTRACT:

A method of improving the nutritional status and reversing the characteristic diarrhea and inflammatory condition in a mammalian creature having ulcerative colitis or inflammation of the colon which contains in combination (a) an oil blend which contains eicosapentaenoic acid (20:5n3) and/or docosahexaenoic acid (22:6n3), and (b) a source of indigestible carbohydrate which is metabolized to short chain fatty acids by microorganisms present in the human colon. Preferably the nutritional product also contains one or more nutrients which act as antioxidants.

19 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

In the first experiment the objective was to determine short chain fatty acid production from a variety of indigestible oligosaccharides during fermentation with mixed human fecal microbiota. Several indigestible oligosaccharides were tested including FOS, Raftilose.RTM. and XOS. FOS is a fructooligosaccharide produced on a commercial scale by fermenting granulated sucrose in water with a pure strain of *Aspergillus niger*. The organism produces a fructosyltransferase enzyme which links additional fructose units onto the fructose end of sucrose molecules to produce 1-kestose (GF.sub.2), nystose (GF.sub.3) and 1.sup.F

- .beta.-fructo-furanosylmaltose (GF.sub.4). Raftilose.RTM. is a fructooligosaccharide **produced via enzymatic hydrolysis of inulin**, which is marketed by Rhone-Poulenc (Raffinerie Tirlemontoise SA). The hydrolysis results in a wide array of oligosaccharides such as GF.sub.2, GF.sub.3 and GF.sub.4 as well as oligosaccharides containing just fructose (F.sub.3, F.sub.4, F.sub.5, etc.). XOS is a xylooligosaccharide produced via enzymatic hydrolysis of xylan. The primary ingredients of XOS are xylobiose, xylotriose and xylotetrose.

US-PAT-NO: 5380661

DOCUMENT-IDENTIFIER: US 5380661 A

TITLE: Bacillus licheniformis NRRL B-18962 capable of producing levan sucrase in the absence of sucrose

DATE-ISSUED: January 10, 1995

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|---------|-------|----------|---------|
| Charles; Robert L. | Elkhart | IN | N/A | N/A |
| Shetty; Jayarama K. | Elkhart | IN | N/A | N/A |

APPL-NO: 08/ 138004

DATE FILED: October 19, 1993

PARENT-CASE:

This is a divisional of application Ser. No. 07/884,183, filed May 18, 1992, now U.S. Pat. No. 5,334,524.

US-CL-CURRENT: 435/252.5; 435/836

ABSTRACT:

The invention relates to an acid stable levan sucrase derived from Bacillus licheniformis NRRL B-19862. The levan sucrase is produced by culturing Bacillus licheniformis NRRL B-19862 in a suitable nutrient medium but in the absence of sucrose. The invention also discloses compositions containing the levan sucrase.

2 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

TITLE - TI:

Bacillus licheniformis NRRL B-18962 capable of producing levan sucrase in the absence of sucrose

Abstract Text - ABTX:

The invention relates to an acid stable levan sucrase derived from *Bacillus licheniformis* NRRL B-19862. The levan sucrase is produced by culturing *Bacillus licheniformis* NRRL B-19862 in a suitable nutrient medium but in the absence of sucrose. The invention also discloses compositions containing the levan sucrase.

Brief Summary Text - BSTX:

The present invention relates to an acid stable levan sucrase enzyme, microorganisms producing it, a process for the preparation of this acid stable levan sucrase enzyme and compositions containing it.

Brief Summary Text - BSTX:

Levan sucrase enzymes have been isolated from a variety of microbial sources, microorganisms such as *Acetobacter suboxydans*, *Actinomyces viscosus*, *Aerobacter levanicum*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, *Bacillus mesentericus*, *Bacillus subtilis*, *Glucobacter oxydans*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptomyces griseus*, *Zymomonas mobilis*. In most cases, the enzyme is extracellular and heat labile. One of the most important features in the production of levan sucrase enzymes is that at least 5% sucrose must be added to the growth medium in order to induce the synthesis of the enzyme using known levan sucrase enzymes producing microorganisms.

Brief Summary Text - BSTX:

As a result, it becomes difficult to separate levan sucrase enzyme from the culture broth because the viscosity of the culture broth increases as levan is produced during fermentation. So there is always a constant search for microorganisms requiring low levels or no sucrose for the production of levan sucrase.

Brief Summary Text - BSTX:

Japanese Patent Application JP-A-52-82781 discloses a method for producing an extracellular levan sucrase enzyme in the presence of low concentrations of sucrose (0.3% weight/volume) using *Bacillus licheniformis* strain AJ 3982 (Institute of Microbial Engineering deposition No. 3373). However this enzyme still needs sucrose to be induced and produced. Moreover this levan sucrase enzyme does not develop any enzymatic activity at a pH of 4.0 when held at a temperature of 55.degree. C. At said temperature it develops only 50% of its maximum activity at a pH of about 5.2 and about 80% of its maximum activity at a pH from about 6 to about 7.8. This narrow pH range restricts the field of application of this levan sucrase enzyme. Moreover acid stability of the levan sucrase enzyme is very important for widespread commercial application of the enzyme.

Brief Summary Text - BSTX:

The present invention also provides a process for the **production of an acid stable levan sucrase** enzyme using a microorganism in the absence of added sucrose to the nutrient medium.

Brief Summary Text - BSTX:

The process according to the invention for the **production of an acid stable levan sucrase** enzyme comprises the following steps of:

Brief Summary Text - BSTX:

The present invention also aims to provide a new microorganism belonging to the genus Bacillus **producing an acid stable levan sucrase** enzyme in the absence of sucrose in the nutrient medium.

Brief Summary Text - BSTX:

For this purpose the present invention provides a new microorganism of the species Bacillus licheniformis **producing an acid stable levan sucrase** enzyme in the absence of sucrose in the nutrient medium. The preferred microorganism of the species Bacillus licheniformis has been deposited with the Agricultural Research Culture Collection (NRRL) Peoria, Ill. under the Budapest Treaty.

Brief Summary Text - BSTX:

The **levan sucrase enzyme of the present invention can be produced** not only by the strain of Bacillus licheniformis APMC 84 but also by natural or artificial mutants and other derivatives of this microorganism. Such mutants can be obtained by well-known techniques, such as X-ray, ultraviolet irradiation, chemical mutagens and genetic engineering.

Brief Summary Text - BSTX:

The **levan sucrase enzyme produced** in accordance with the process of the invention is prepared by cultivating the microorganism belonging to the genus Bacillus in a nutrient medium containing carbon, nitrogen and inorganic salts under aerobic conditions in the absence of sucrose and then recovering the levan **sucrase** enzyme therefrom.

Detailed Description Text - DETX:

The medium used for the **production of levan sucrase** enzyme is prepared with the following components: corn syrup 10.3% weight/volume sold under the trademark

STALEY 200 com syrup (A. E. STALEY), soybean flour 5.5% sold under the trademark PROMOSOY 100 (CENTRAL SOYA), yeast extract (sold by UNIVERSAL FOODS CORP) 0.32%, sodium monohydrogen phosphate 0.7% and sodium dihydrogen phosphate 0.7%.

Detailed Description Text - DETX:

The rate of formation of glucose from sucrose by levan sucrase enzyme increased with increasing concentration of the enzyme. An amount of 35% levan was produced in all the cases. A amount of 45% glucose was produced in all the cases.

Claims Text - CLTX:

2. A mutant of the Bacillus licheniformis, is NRRL B-18962 wherein said mutant is capable of producing levan sucrase in the absence of sucrose.

US-PAT-NO: 5380657

DOCUMENT-IDENTIFIER: US 5380657 A

TITLE: Method for isolation of insertion elements from coryneform bacteria

DATE-ISSUED: January 10, 1995

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-----------|-------|----------|---------|
| Schaefer; Andreas | Bielefeld | N/A | N/A | DE |
| Seep-Feldhaus; | Bielefeld | N/A | N/A | DE |
| Anna-Hildegard | Bielefeld | N/A | N/A | DE |
| Jaeger; Wolfgang | Bielefeld | N/A | N/A | DE |
| Kalinowski; Joern | Bielefeld | N/A | N/A | DE |
| Wohlleben; Wolfgang | Bielefeld | N/A | N/A | DE |
| Puehler; Alfred | | | | |

APPL-NO: 08/ 033320

DATE FILED: March 18, 1993

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|----------------|
| DE | 4208785 | March 19, 1992 |

US-CL-CURRENT: 435/6; 435/252.32 ; 435/320.1 ; 435/473 ; 435/487

ABSTRACT:

A method locating insertion elements (IS elements) or transposons in coryneform bacteria, a positive selection system suitable for the above, the IS elements found in this manner and their use, is disclosed. The method involves:

(1) The construction of a non-self-transferrable vector mobilizable from an E. coli mobilizer strain which vector is composed of

- (a) A DNA segment containing a replicon functional in E. coli,
 - (b) A second DNA segment containing the DNA fragment coding for the mobilization function (Mob site containing the oriT),
 - (c) A third DNA segment which recombines homologously in Gram-positive bacteria and/or contains a replicon functional in coryneform bacteria,
 - (d) A DNA segment from Bacillus subtilis containing the sacB gens,
- (2) Transfer of this vector by means of conjugative transfer into the coryneform recipient strains,

(3) Cultivation of the transconjugants containing the vector in an about 10% sucrose-containing nutrient medium,

(4) Lysis of the sucrose-resistant clones, cleaving of the plasmids with restriction endonucleases and analysis of the fragments.

4 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Detailed Description Text - DETX:

The sacB gene from *Bacillus subtilis* is used for the isolation of insertion sequences (IS elements) or transposons in accordance with the invention (Gay et al., J. Bacteriol. (1983), volume 153, pages 1424-1431). The gene codes for the exoenzyme levan sucrase, which catalyzes the reactions of saccharose hydrolysis and levan synthesis (Dedonder et al., Methods in Enzymol. (1966), volume 8, pages 500-505). The expression of sacB in *E. coli* results in the transport of the enzyme into the periplasma (Steinmetz et al., Mol. Gen. Genet. (1983), volume 191, pages 138-144) and is lethal for *E. coli* and other Gram-negative bacteria placed on media containing over 5% sucrose (Gay et al., J. Bacteriol., volume 194, pages 918-921).

Detailed Description Text - DETX:

The sacB gene from *Bacillus subtilis* is used for the isolation of insertion sequences (IS elements) or transposons (Gay et al., J. Bacteriol. (1983), volume 153, pages 1424-1431). The gene codes for the exoenzyme levan sucrase, which catalyses the reactions of saccharose hydrolysis and levan synthesis (Dedonder et al., Methods in Enzymol. (1966), volume 8, pages 500-505). The expression of sacB in *E. coli* results in the transport of the enzyme into the periplasma (Steinmetz et al., Mol. Gen. Genet. (1983), volume 191, pages 138-144) and is lethal for *E. coli* and other Gram-negative bacteria on media with over 5% sucrose (Gay et al., J. Bacteriol., volume 164, pages 913-921).

US-PAT-NO: 5334524

DOCUMENT-IDENTIFIER: US 5334524 A

TITLE: Process for producing levan sucrase using Bacillus licheniformis

DATE-ISSUED: August 2, 1994

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|---------|-------|----------|---------|
| Charles; Robert L. | Elkhart | IN | N/A | N/A |
| Shetty; Jayarama K. | Elkhart | IN | N/A | N/A |

APPL-NO: 07/ 884183

DATE FILED: May 18, 1992

US-CL-CURRENT: 435/193; 424/94.5 ; 435/183 ; 435/252.5 ; 435/836

ABSTRACT:

The present invention relates to an acid stable levan sucrase enzyme which is not induced by sucrose.

The present invention relates to a process for the preparation of this enzyme and microorganisms producing the levan sucrase enzyme. The invention also provides compositions containing this enzyme.

11 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

----- KWIC -----

TITLE - TI:

Process for producing levan sucrase using Bacillus licheniformis

Abstract Text - ABTX:

The present invention relates to a process for the preparation of this enzyme and microorganisms producing the levan sucrase enzyme. The invention also provides compositions containing this enzyme.

Brief Summary Text - BSTX:

The present invention relates to an acid stable levan sucrase enzyme, micro organisms producing it, a process for the preparation of this acid stable levan sucrase enzyme and compositions containing it.

Brief Summary Text - BSTX:

Levan sucrase enzymes have been isolated from a variety of microbial sources, microorganisms such as Acetobacter suboxydans, Actinomyces viscosus, Aerobacter levanicum, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus mesentericus, Bacillus subtilis, Glucobacter oxydans, Streptococcus mutans, Streptococcus salivarius, Streptomyces griseus, Zymomonas mobilis. In most cases, the enzyme is extracellular and heat labile. One of the most important features in the production of levan sucrase enzymes is that at least 5% sucrose must be added to the growth medium in order to induce the synthesis of the enzyme using known levan sucrase enzymes producing microorganisms.

Brief Summary Text - BSTX:

As a result, it becomes difficult to separate levan sucrase enzyme from the culture broth because the viscosity of the culture broth increases as levan is produced during fermentation. So there is always a constant search for microorganisms requiring low levels or no sucrose for the production of levan sucrase.

Brief Summary Text - BSTX:

Japanese Patent Application JP-A-52-82781 discloses a method for producing an extracellular levan sucrase enzyme in the presence of low concentrations of sucrose (0.3% weight/volume) using Bacillus licheniformis strain AJ 3982 (Institute of Microbial Engineering deposition No. 3373). However this enzyme still needs sucrose to be induced and produced. Moreover this levan sucrase enzyme does not develop any enzymatic activity at a pH of 4.0 when held at a temperature of 55.degree. C. At said temperature it develops only 50% of its maximum activity at a pH of about 5.2 and about 80% of its maximum activity at a pH from about 6 to about 7.8. This narrow pH range restricts the field of application of this levan sucrase enzyme. Moreover acid stability of the levan sucrase enzyme is very important for widespread commercial application of the enzyme.

Brief Summary Text - BSTX:

The present invention also provides a process for the production of an acid stable levan sucrase enzyme using a microorganism in the absence of added sucrose to the nutrient medium.

Brief Summary Text - BSTX:

The process according to the invention for the **production of an acid stable levan sucrase** enzyme comprises the following steps of:

Brief Summary Text - BSTX:

The present invention also aims to provide a new microorganism belonging to the genus *Bacillus* **producing an acid stable levan sucrase** enzyme in the absence of sucrose in the nutrient medium.

Brief Summary Text - BSTX:

For this purpose the present invention provides a new microorganism of the species *Bacillus licheniformis* **producing an acid stable levan sucrase** enzyme in the absence of sucrose in the nutrient medium. The preferred microorganism of the species *Bacillus licheniformis* has been deposited with the Agricultural Research Culture Collection (NRRL), Peoria, Ill., under the Budapest Treaty.

Brief Summary Text - BSTX:

The **levan sucrase enzyme of the present invention can be produced** not only by the strain of *Bacillus licheniformis* APMC 84 B-1896, but also by natural or artificial mutants and other derivatives of this microorganism. Such mutants can be obtained by well-known techniques, such as X-ray, ultraviolet irradiation, chemical mutagens and genetic engineering.

Brief Summary Text - BSTX:

The **levan sucrase enzyme produced** in accordance with the process of the invention is prepared by cultivating the microorganism belonging to the genus *Bacillus* in a nutrient medium containing carbon, nitrogen and inorganic salts under aerobic conditions in the absence of sucrose and then recovering the levan **sucrase** enzyme therefrom.

Detailed Description Text - DETX:

The medium used for the **production of levan sucrase** enzyme is prepared with the following components: corn syrup 10.3% weight/volume sold under the trademark STALEY 200 corn syrup (A. E. STALEY), soybean flour 5.5% sold under the trademark PROMOSOY 100 (CENTRAL SOYA), yeast extract (sold by UNIVERSAL FOODS CORP) 0.32%, sodium monohydrogen phosphate 0.7% and sodium dihydrogen phosphate 0.7%.

Detailed Description Text - DETX:

The rate of formation of glucose from sucrose by levan sucrase enzyme increased with increasing concentration of the enzyme. An amount of 35% levan was produced in all the cases. A amount of 45% glucose was produced in all the cases.

Claims Text - CLTX:

1. A levan sucrase enzyme produced by Bacillus licheniformis APMC 84 having deposit number NRRL B-18962 and mutants thereof capable of producing said levan sucrase enzyme wherein said enzyme possesses at a temperature of about 55 C. a maximum activity at a pH of about 5.5 and retains at least 50% of its maximum activity at a pH of 4.0, as determined by the amount of glucose produced under the assay conditions which include sucrose as a substrate and measurement by high performance liquid chromatography.

Claims Text - CLTX:

2. The levan sucrase enzyme of claim 1 wherein said enzyme is produced in the absence of sucrose.

Claims Text - CLTX:

11. A levan sucrase enzyme produced by Bacillus licheniformis APMC 84, having deposit number NRRL B-18962, and mutants thereof capable of producing said levan sucrase enzyme, wherein said enzyme possesses at a temperature of about 55 C. a maximum activity at a pH of about 5.5 and retains at least 50% of its maximum activity at a pH of 4.0, as determined by the amount of glucose produced under assay conditions which include sucrose as a substrate and measurement by high performance liquid chromatography, and wherein said levan sucrase enzyme is produced by a process comprising the steps of:

US-PAT-NO: 5310647

DOCUMENT-IDENTIFIER: US 5310647 A

TITLE: Detection and measurement of destructive and polymer forming enzymes by colloidal flocculation

DATE-ISSUED: May 10, 1994

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------------|----------------|-------|----------|---------|
| Kerschensteiner; Daniel | Chester County | PA | N/A | N/A |

A.

APPL-NO: 07/ 861654

DATE FILED: April 1, 1992

US-CL-CURRENT: 435/4; 435/23 ; 435/962 ; 436/811

ABSTRACT:

The change between a dispersed state and a flocculated state of a colloidal agent (e.g. Congo Rubin, colloidal gold) is used to provide sensitive visual detection and optionally assay of enzyme in a sample. The test cell includes a substrate for the enzyme, and, depending on the action of the enzyme, polymer which protects the colloid from electrolyte-induced flocculation is either formed or destroyed by the test reaction. In a test for a hydrolytic enzyme (e.g. a protease) the colloid loses protection and flocculates. In a test for a polymer-forming enzyme, the colloid gains protection and is prevented from flocculating by added electrolyte, or alternatively, depending on the polymer's behavior, becomes more sensitive to the flocculating action of the added electrolyte. The measurement may be quantitated using an instrumental monitor. The test uses a natural substrate for the enzyme to be assayed (e.g., gelatin for the gelatinase class of enzymes) and may achieve rapid speed. The test may be performed using a biologically-derived sample for diagnostically relevant purposes, e.g. to detect an enzyme indicative of periodontal disease. A kit for performing the test is also disclosed.

7 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Detailed Description Text - DETX:

The overall concept of the present invention is also applicable to the reverse

situation, that of measuring enzyme activity responsible for the synthesis, instead of the cleaving, of polymers from simple precursors or monomers. In one example of this reverse application, very small amounts of polysaccharide--a glucan or levan--were measured as a result of synthesis by glucosyl and fructosyl transferases from the disaccharide sucrose by a streptococcal species associated with plaque production and with caries risk. Other examples of enzymes that can be detected in this manner include DNA and RNA polymerases, viral reverse transcriptases, and glycogen synthase.

Detailed Description Text - DETX:

Streptococcus salivarius is known to produce a water-insoluble dextran--a polymer composed entirely of glucose residues--in addition to a water-soluble levan--a polymer of fructose--produced enzymatically by action of glucosyl and fructosyl transferases, respectively utilizing the substrate sucrose. Some of these extracellular polysaccharides ("ECP's") produced by this species and mutants streptococci are thought to be responsible for initiation of plaque conditions and therefore might be associated with the risk of dental decay (Scheie and Rolla, 1984, 1986).

US-PAT-NO: 5300431

DOCUMENT-IDENTIFIER: US 5300431 A

TITLE: Positive selection vector for the bacteriophage P1 cloning system

DATE-ISSUED: April 5, 1994

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|--------------|-------|----------|---------|
| Pierce; James C. | Wilmington | DE | N/A | N/A |
| Sternberg; Nat L. | West Chester | PA | N/A | N/A |

APPL-NO: 07/ 662224

DATE FILED: February 26, 1991

US-CL-CURRENT: 435/6; 435/252.33 ; 435/320.1 ; 536/23.2 ; 536/24.1

ABSTRACT:

Positive selection cassettes are disclosed which contain a lethal gene, a promoter, a repressor sequence overlapping the promoter, and a cloning site between the promoter and the lethal gene. Insertion of a foreign nucleic acid sequence into the cloning site prevents expression of the lethal gene. Expression of the lethal gene under nonrepressed conditions kills a host organism containing a positive selection cassette which does not contain the foreign nucleic acid sequence.

5 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

----- KWIC -----

Brief Summary Text - BSTX:

Another positive selection system used in DNA cloning is based on the sacB gene from *Bacillus subtilis*. This gene codes for the enzyme levansucrase (sucrose:2,6-.beta.-D-fructan 6-.beta.-D fructosyltransferase; EC 2.4.1.10) which catalyzes the transfructorylation of sucrose to various acceptor substrates resulting in the hydrolysis of sucrose and levan synthesis. Gay et al., J. Bacteriol. 164, 918-921 (1985) demonstrated that the production of levansucrase in *E. coli* is lethal in the presence of growth media containing 5% sucrose. Gay et al. have used this knowledge to develop a positive selection cloning system based on inactivating the *B. subtilis* sacB structural gene.

This allows the growth of only those *E. coli* bacteria containing recombinant clones that have DNA inserts when grown in the presence of sucrose.

US-PAT-NO: 5223431

DOCUMENT-IDENTIFIER: US 5223431 A

TITLE: Leuconostoc Dextranicum NRRL-B-18242

DATE-ISSUED: June 29, 1993

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-------------|-------|----------|---------|
| Pucci; Michael J. | New Britain | CT | N/A | N/A |
| Kunka; Blair S. | Bradenton | FL | N/A | N/A |

APPL-NO: 07/ 393211

DATE FILED: August 14, 1989

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This is a divisional of copending application Ser. No. 07/243,677 filed on Sept. 13, 1988, now U.S. Pat. No. 4,933,191 which is a continuation-in-part of U.S. patent application Ser. No.68,435, filed Jul. 1, 1987, U.S. Pat. No. 4,855,14

US-CL-CURRENT: 435/252.9; 435/103 ; 435/853

ABSTRACT:

Leuconostoc dextranicum NRRL-B-18242 which produces a slushy, applesauce-like dextran with a particulate structure is described. The dextran is dried and used in foods and the like.

5 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Brief Summary Text - BSTX:

Dextrans are glucose polymers synthesized by several genera of bacteria including Streptococcus, Lactobacillus and Leuconostoc (Schwartz, R. D. and E. A. Bodie, Appl Environ Microbiol. 48:678-679 (1984); and Lawford, G. R., A. Kligerman and T. Williams, Biotechnol. Bioeng. 21:1121-1131 (1979)). Primarily extracellular dextranucrase enzymes synthesize dextrans with molecular weights of 2.times.10.sup.7 and higher (Schwartz, R. D. and E. A.

Bodie, Appl. Environ. Microbiol. 48:678-679 (1984)). The dextrans are primarily alpha 1,6 linked, but may also have alpha 1,4, alpha 1,2, and alpha 1,3 linkages which result in branched polymers of varying water solubilities and other properties (Niinobe, M. and T. Kobayashi, Nippon Nogeikagaku Kaishi 46:81-88 (1972)). Dextrans are presently used in a variety of industries, however, uses in foods are non-existent.

US-PAT-NO: 5089401

DOCUMENT-IDENTIFIER: US 5089401 A

TITLE: Method for the preparation of fructose-containing oligosaccharide

DATE-ISSUED: February 18, 1992

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|----------|-------|----------|---------|
| Fujita; Koki | Osaka | N/A | N/A | JP |
| Hara; Kozo | Yokohama | N/A | N/A | JP |
| Hashimoto; Hitoshi | Kamakura | N/A | N/A | JP |
| Kitahata; Sumio | Sennan | N/A | N/A | JP |

APPL-NO: 07/ 672388

DATE FILED: March 20, 1991

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|----------------|
| JP | 2-207582 | August 7, 1990 |

US-CL-CURRENT: 435/97; 435/100 ; 435/101 ; 435/830 ; 536/127 ; 536/25.3

ABSTRACT:

An enzymatic method for the preparation of a fructose-containing oligosaccharide, in which a .beta.-fructofuranosidase obtained by culturing *Arthrobacter* sp. K-1 (FERM BP-3192) as an enzyme is reacted on sucrose, raffinose or stachyose as the donor in the presence of an aldose or ketose as the receptor. The enzyme is characterized by:

(1) activity on sucrose for the transglycosidation of a fructosyl group to the receptor in the presence of a monosaccharide, sugar alcohol, alkyl alcohol, glycoside or oligosaccharide;

(2) activity for the decomposition of sucrose, elrose, neokestose, xylsucrose, raffinose and stachyose with inactivity on a saccharide selected from the group consisting of 1-kestose, nistose, inulobiose and levan biose;

(3) optimum pH of 6.5 to 6.8 at 40.degree. C. with stability at a pH of 5.5 to 10;

(4) optimum temperature of 55.degree. C. at a pH of 6.5 exhibiting at least 70% of residual activity at 60.degree. C.;

(5) susceptibility to inhibition by the ions of silver, mercury, zinc, copper and tin;

(6) two molecular weights of 52,000. \pm 2,500 and 58,000. \pm 2,500; and

(7) two isoelectric points of pH 4.3 and pH 4.6.

15 Claims, 9 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 9

----- KWIC -----

Brief Summary Text - BSTX:

Known transferases of fructosyl groups include the levan sucrase produced by *Bacillus subtilis* and β -fructofuranosidase produced by the fungi such as *Aspergillus niger*, *Penicillium oxalicum*, *Penicillium frequentans*, *Penicillium* sp. K25 and the like. It is also known that xylsucrose and isomaltosyl fructoside synthesized by utilizing the activity of levan sucrase among them for transglycosidation are characteristically cariostatic and lactosucrose has an activity as a growth factor of bifidus bacteria so that these oligosaccharides have a potentiality for practical application in the future as a functional saccharide. Since the levan sucrase used in the production of these oligosaccharides is derived from sucrose, it is indispensable that the culture medium contains sucrose so that levan is unavoidably formed in the liquid culture medium resulting in an increased viscosity of the medium to cause a difficulty in handling of the medium. As is pointed out, in addition, this enzyme has problems in respects of the low productivity, poor heat resistance and so on. Further, the β -fructofuranosidase produced by conventional fungi is an endoenzyme of the fungal body and is defective in the narrow receptor specificity.

Detailed Description Text - DETX:

This reaction mixture was subjected to column chromatographic purification. Thus, a chromatographic column of active charcoal was loaded with the reaction mixture and, after washing with water to remove monosaccharides, elution of the sucrose fraction was performed with 5% ethyl alcohol as the eluant. Thereafter, elution was continued with 20% ethyl alcohol and the eluate fraction thus obtained was concentrated by evaporation followed by freeze-drying to give 7 g of a powder which was the transglycosidation product A having a purity of 99%. This transglycosidation product A could be hydrolyzed with an acid to form fructose and xylose and it was found by the β -fructofuranosidase enzymolysis using *Saccharomyces* that the molar ratio of fructose and xylose was 1:1. This transglycosidation product had no reducing activity and good coincidence was obtained thereof with the transglycosidation product prepared by using levan sucrase relative to the retention time in the high-performance liquid chromatography and the R_f value in the paper chromatography. FIG. 1 shows a ^{13}C -NMR spectrum of this transglycosidation product A.

US-PAT-NO: 4933191

DOCUMENT-IDENTIFIER: US 4933191 A

TITLE: Novel dextran produced by leuconostoc dextranicum

DATE-ISSUED: June 12, 1990

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-------------|-------|----------|---------|
| Pucci; Michael J. | New Britain | CT | N/A | N/A |
| Kunka; Blair S. | Bradenton | FL | N/A | N/A |

APPL-NO: 07/ 243677

DATE FILED: September 13, 1988

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of application Ser. No. 68,435 , now U.S. Pat. No. 4,855,149, filed July 1,1987.

US-CL-CURRENT: 426/48; 426/531 ; 426/61 ; 426/657 ; 426/658 ; 435/103

ABSTRACT:

A novel dextran having a slushy, applesauce-like appearance with a particulate gel-like structure in crude form is described. The dextran is particularly produced by *Leuconostoc dextranicum* NRRL-B-18242. The dextran in particulate or dried form is used in foods and other applications where texture is important.

17 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

----- KWIC -----

Brief Summary Text - BSTX:

Dextrans are glucose polymers synthesized by several genera of bacteria including *Streptococcus*, *Lactobacillus* and *Leuconostoc* (Schwartz, R.D. and E. A. Bodie, Appl. Environ. Microbiol. 48:678-679 (1984); and Lawford, G.R., A. Kligerman and T. Williams, Biotechnol. Bioeng. 21:1121-1131 (1979)). Primarily extracellular dextranucrase enzymes synthesize dextrans with

molecular weights of 2×10^7 and higher (Schwartz, R.D. and E. A. Bodie, Appl. Environ. Microbiol. 48:678-679 (1984)). The dextrans are primarily alpha 1,6 linked, but may also have alpha 1,4, alpha 1,2, and alpha 1,3 linkages which result in branched polymers of varying water solubilities and other properties (Niinobe, M. and T. Kobayashi, Nippon Nogeikagaku Kaishi 46:81-88 (1972)). Dextrans are presently used in a variety of industries, however, uses in the foods are non-existent.

US-PAT-NO: 4927811

DOCUMENT-IDENTIFIER: US 4927811 A

TITLE: Method and composition for improved animal husbandry

DATE-ISSUED: May 22, 1990

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|--------------|-------|----------|---------|
| Quarles; Carey L. | Fort Collins | CO | N/A | N/A |

APPL-NO: 07/ 271931

DATE FILED: November 15, 1988

US-CL-CURRENT: 514/23; 424/442 ; 426/2 ; 426/658 ; 514/54 ; 514/867

ABSTRACT:

The present invention relates to a method and composition for increasing the breast weight to body weight ratio in poultry. The present invention also relates to methods and compositions for reducing mortality and reducing the occurrence of air sac lesions in poultry and livestock. The compositions of the present invention include fructooligosaccharides, and in particular, fructooligosaccharides produced by the action of a fructosyl transferase enzyme on a sucrose substrate. The methods of the present invention involve the administration of compositions including fructooligosaccharides to poultry and livestock. The fructooligosaccharides are preferably administered by incorporation into the poultry or livestock's food supply.

18 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Certain fungi, such as, *Aspergillus* and *Aureobasidium*, as well as other microorganisms are known to produce the enzyme fructosyl transferase. Fructosyl transferases which produce oligosaccharides are also known to be produced by chicory plant and by onion plant. See Singh et al., Substrate Specificity of Fructosyl Transferase From Chicory Roots, *Phytochemistry* vol. 10, pp. 2037-39 (1971) and Henry et al., Sucrose: Sucrose Fructosyltransferase and Fructan:Fructan Fructosyltransferase From *Allium Cepa*, *Phytochemistry* vol. 19, pp. 1017-20 (1980). The present invention, however, is not intended to be limited to the production of fructooligosaccharides by a fruct syl transferase

from any particular source of microorganism or plant. In addition, fructooligosaccharides for use in the present invention can be produced by chemical methods. For example, U.S. Pat. No. 4,613,377 to Yamazaki, et al. describes a process for producing fructooligosaccharides by the hydrolysis of inulin by heating an aqueous solution containing inulin at a temperature of about 70.degree. C. to about 100.degree. C. U.S. Pat. No. 4,613,377 is incorporated herein by reference.

US-PAT-NO: 4927757

DOCUMENT-IDENTIFIER: US 4927757 A

TITLE: Production of substantially pure fructose

DATE-ISSUED: May 22, 1990

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|-------------|-------|----------|---------|
| Hatcher; Herbert J. | Idaho Falls | ID | N/A | N/A |
| Gallian; John J. | Twin Falls | ID | N/A | N/A |
| Leeper; Stephen A. | Idaho Falls | ID | N/A | N/A |

APPL-NO: 07/ 225914

DATE FILED: July 29, 1988

US-CL-CURRENT: 435/105; 435/101 ; 435/175 ; 435/176 ; 435/193 ; 435/813
; 435/94 ; 435/97 ; 435/99

ABSTRACT:

A process is disclosed for the production of substantially pure fructose from sucrose-containing substrates. The process comprises converting the sucrose to levan and glucose, purifying the levan by membrane technology, hydrolyzing the levan to form fructose monomers, and recovering the fructose.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Detailed Description Text - DETX:

In accordance with the subject invention, a process for the **production of essentially pure levan** or crystalline fructose and high fructose syrup, greater than 60% fructose, is provided. The instant process offers advantages over other practiced methods for the production of fructose as being both energy and cost efficient. First, any source of sucrose which is freed of solid matter can be utilized to form levan and glucose by the action of a fructosyl transferring enzyme, such as levan **sucrase**. Additionally, as opposed to current methods for the production of fructose which are very energy intensive due to evaporation procedures, the current invention greatly reduces processing cost for the production of fructose by a reduced need for evaporation because of initial concentration of the product by membrane technology. Membrane technology includes any method for purification or concentration by filtering

such as ultrafiltration, hyperfiltration, diafiltration, etc.

Detailed Description Text - DETX:

Sucrose is converted to levan and glucose by the action of the fructosyl transferring enzyme. Fructosyl transferring enzyme refers to any enzyme that catalyzes transfructosylation, or the transfer of a fructose moiety of sucrose to another molecule (such as **levansucrase**). A specific reaction yields levan, a polysaccharide comprising 2 or more fructosyl units per molecule. Many such enzyme preparations are known including the enzyme prepared from *Microbacterium laevaniformans*, ATCC No. 15953. See also, Adriaan Fuchs, "On the **synthesis and breakdown of levan** by bacteria," Doctoral Thesis, University of Leiden, 1959.

Detailed Description Text - DETX:

An example of the polymerization subprocess is the converting of sucrose to levan and glucose by the enzyme **levansucrase**. Since one of the byproducts, glucose, inhibits the **synthesis of levan**, continuous removal from the reaction can be beneficial. Glucose removal can be achieved by the use of bioreactor designs which provide for continuous byproduct removal. Such designs include, but are not limited to: (1) continuously stirred tank reactors (CSTR); (2) hollow-fiber membrane bioreactors (HFBR); and (3) coupled-CSTR-ultrafiltration-recycle bioreactors (CUFBR). Options (2) and (3) can be operated in continuous, semi-batch, or fed-batch modes. Although all three bioreactors provide for the removal of the inhibiting glucose byproduct, for the purposes of the present invention, the HFBR or CUFBR bioreactors are preferred as both systems allow the enzyme preparation and intermediates to be retained at an enhanced level relative to the byproducts. In these reactors, the cells and polymers are retained by a membrane while the glucose is removed from the system in a waste stream. A retentate stream consisting essentially of levan is collected. A portion of the retentate stream may be recycled into the bioreactor to utilize unreacted sucrose.

US-PAT-NO: 4927756

DOCUMENT-IDENTIFIER: US 4927756 A

TITLE: Water soluble iron dextran and a process for its manufacture

DATE-ISSUED: May 22, 1990

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|----------|-------|----------|---------|
| Schwengers; Dieter | Dormagen | N/A | N/A | DE |

APPL-NO: 07/ 159146

DATE FILED: February 25, 1988

PARENT-CASE:

This is a division of application Ser. No. 741,395, filed June 5, 1985, now U.S. Pat. No. 4,749,695.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| DE | 3422249 | June 15, 1984 |

US-CL-CURRENT: 435/103; 435/97 ; 536/112 ; 536/113 ; 536/114

ABSTRACT:

A water-soluble iron dextran having a high iron content is prepared by adding to an aqueous solution containing more than 200 mmol D-glucose per 1000 U.alpha.(1.fwdarw.6)-D-glucosyltransferase, at 265 to 310 K and a pH value of 4.5 to 8, an aqueous sucrose solution in a mole ratio of sucrose to glucose of from 2.0 to 5.0; separating, after the consumption of the sucrose, glucose, liberated fructose and undesired oligosaccharides; reacting the so-purified dextran, having an average molar mass of from 2000 to 4000, with freshly precipitated iron(III) hydroxide and, if desired, further purifying the same.

Iron dextrans having an iron content of from 27 to 33 percent by weight and an average molar mass of the dextran component of from 2000 to 4000, may be prepared according to the process of the invention which can be used in the treatment of iron deficiency.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

According to the classification of the "Enzyme Commission", enzymes which transfer the D-glucopyranosyl group of sucrose to suitable acceptors are designated as α -(1 \rightarrow 6)-D-glucosyl transferase. An extracellular enzyme of the kind is dextran sucrase (E.C. 2.4.1.5) which is formed by specific kinds of bacteria of the lactobacilli species, for example, *Leuconostoc mesenteroides*, in particular the strain B-512, *Leuconostoc dextranicum*, *Streptococcus* and *lactobacillus*. When preparing native dextran, sucrose serves primarily as acceptor and acts as chain initiator for a chain polymerization in which by virtue of continuous transfer of D-glucopyranosyl groups from the sucrose to the growing chain of the polysaccharide, dextrans having molar masses of several millions are formed, while, at the same time, a fructose molecule is liberated for each reacted molecule of sucrose.

US-PAT-NO: 4879228

DOCUMENT-IDENTIFIER: US 4879228 A

TITLE: Microbial production of polyfructose

DATE-ISSUED: November 7, 1989

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------|----------|-------|----------|---------|
| Mays; Thomas D. | Columbia | MD | N/A | N/A |
| Dally; Ellen L. | Columbia | MD | N/A | N/A |

APPL-NO: 06/ 689238

DATE FILED: January 4, 1985

US-CL-CURRENT: 435/101; 435/252.1 ; 435/822

ABSTRACT:

A water-soluble levan having a weight average molecular weight of about 10,000-40 million, preferably about 5-25 million and especially about 10-20 million which stabilizes a bovine serum albumin colloid having index (EAI) of about 3-100 determined according to the formula $EAI = 2T/OC$ wherein T is turbidity measured at 500 nm, C is the weight of emulsified protein per unit volume of the aqueous phase, and O is the volume fraction of a dispersed oil phase is produced by fermenting a nutrient growth medium feedstock having a carbon source consisting essentially of an assimilable sugar selected from the group consisting of sucrose, raffinose, or a mixture thereof with a *Zymomonas mobilis* microorganism capable of converting at least 50% of the fructose value of the sugar to said levan under nutrient growth conditions which enhance levan production while suppressing ethanol production. It is useful as a colloid stabilizing agent, particularly with foods, beverages pharmaceuticals, dentrifices, and cosmetics.

1 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

Brief Summary Text - BSTX:

This invention relates to a water-soluble, essentially uncharged levan polymer which has useful emulsifying and thickening properties comparable to those of

gum arabic, and to methods for the production and use thereof as well as compositions containing said levan polymer as an emulsifying agent. More particularly, this invention relates to the production of levan through the action of levan sucrase produced by an improved and modified bacterial strain of *Zymomonas mobilis*.

Brief Summary Text - BSTX:

Levan has been used as an immunologic suppressor (*Experientia* 34: 1362-1363, 1978 and *J. Pathol.* 125: 103, 1978) and an immunogenic hapten conjugate (*J. Dent. Res.* 55: 129, 1976 and Moreno, U.S. Patent No. 4,260,602). UK Patent Application GB No. 2,046,757-A published Nov. 19, 1980 describes a low cariogenic food sweetener prepared by subjecting a substrate solution containing aldose and sucrose or raffinose to the activity of levan sucrase, but the desired product is a trisaccharide and not a high molecular weight levan, which has not heretofore been to provide functional properties to food products.

Brief Summary Text - BSTX:

Traditionally, small quantities of levan have been produced by the bacterial fermentation of sucrose, usually by a strain of *Actinomyces viscosus* or *Aerobacter levanicum*. Levan produced by the in vitro activity of levan sucrase has been reported by T. Tanaka et al. in *J. Biochem.* 87 (1): 297-303 (1980). The applications of recombinant DNA technology have been applied in the in vitro genetic transfer of the *sacB* gene from *Bacillus subtilis* into a strain of *Escherichia coli* (*J. Bacteriol.* 153: 1424-31, 1983.) These processes, however, do not represent efficient means for the production of levan. In particular, the in vitro enzymatic synthesis of levan from sucrose would appear to be difficult in terms of producing a high molecular weight molecule, as evidenced by UK Patent Application GB No. 2,046,757-A.

Detailed Description Text - DETX:

The application described herein are based upon the use of a molecule with a weight average molecular weight of 10 to 20 million, as determined by gel filtration chromatography using Bio-Rad "Biogel" agarose having a molecular weight exclusion limit of 15 million using bacterial cells as void markers. It would be difficult to produce such a large molecular weight species through column chromatography of sucrose by an immobilized levan sucrase enzyme. The traditional bacterial fermentation of sucrose by strains of *Aerobacter levanicum* results in only 1.3 to 1.5 grams of levan per 100 milliliters of fermentation broth of the final levan product (*Methods in Enzymology* 8: 161-165, 1966). Production of levan by the bacterial fermentation of 2% sucrose by wildtype (or genetically unaltered) strains of *Zymomonas* results in only 0.2 to 0.3 grams of levan product per 100 milliliters of fermentation broth (*Biochem. J.* 98: 804-812, 1966). This represents the utilization of only 2% of the total sucrose substrate present. However, from Table 1, one can see that an efficient wildtype strain in 15% sucrose produces 2.4 grams/100 ml

compared with genetically modified and selected progeny which produce 3.4, 2.9 and 2.9 grams/ 100 ml respectively.

US-PAT-NO: 4877634

DOCUMENT-IDENTIFIER: US 4877634 A

TITLE: Food product containing novel dried compositions with polysaccharides

DATE-ISSUED: October 31, 1989

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-----------|-------|----------|---------|
| Pucci; Michael J. | Sarasota | FL | N/A | N/A |
| Kunka; Blair S. | Bradenton | FL | N/A | N/A |

APPL-NO: 07/ 238415

DATE FILED: August 31, 1988

PARENT-CASE:

This is a divisional of co-pending application Ser. No. 6/068,435 filed on July 1, 1987; now U.S. Pat. No. 4,855,149.

US-CL-CURRENT: 426/531; 426/565 ; 426/580 ; 426/583 ; 426/602 ; 426/658

ABSTRACT:

Novel dried compositions containing polysaccharides (dextrans and levans) derived using a *Leuconostoc* to ferment sucrose to produce the polysaccharides are described. In particular, dried compositions incorporating milk solids or other drying aids to facilitate drying and rehydration of the polysaccharides are described. The compositions are useful as quality (e.g. texture, stability or thickness) improvers for foods.

7 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Dextrans are glucose polymers synthesized by several genera of bacteria including *Streptococcus*, *Lactobacillus*, and *Leuconostoc* (Schwartz, R. D., and E. A. Bodie, Appl. Environ Microbiol. 48:678-679 (1984); and Lawford, G. R., A. Kligerman, and T. Williams, Biotechnol. Bioeng. 21:1121-1131 (1979)). They are generally referred to as "microbial polysaccharides" (Kirk-Othmer 15:439-447 (1981)) Synthesis occurs via dextran sucrose enzymes which are primarily extracellular. Dextran molecular weights range from

1.5.times.10.sup.4 to 2.times.10.sup.7 and higher (Schwartz, R. D., and E. A. Bodie, Appl Environ Microbiol. 48:678-679 (1984)). Dextrans consist of primarily alpha 1,6 linkages but alpha 1,4, alpha 1,2, and alpha 1,3 bonds are also known (Niinobe, M. and T. Kobayashi, Nippon Nogeikagaku Kaishi 46:81-88 (1972)). The extent of branching affects water solubility and other properties. Dextrans are presently used in a variety of industries; however, although the potential exists, uses of dextrans in the food industry are limited at present because of the difficulty in providing a useful dried product which rehydrates in water to produce a suitably thick solution (Jeanes, A., ACS Symp. Ser. 45:284-298 (1977)).

Brief Summary Text - BSTX:

Dextran synthesis has probably been most widely studied in the genera *Leuconostoc*, particularly in *L. mesenteroides* (Lawford, G. R., A. Kligerman, and T. Williams, Biotechnol. Bioeng. 21:1121-1131 (1979); Niinobe, M. and T. Kobayashi, Nippon Nogeikagaku Kaishi 46:81-88 (1972); and Preobrazhenskaya, M.E. and N. A. Danilova., Prikladnaya Biokhimiya i Mikrobiologiya 10:539-546 (1974)). *Leuconostoc* sp. offer several advantages. First, they are unable to metabolize either dextrans (they contain no dextranases) or sucrose (no invertases or sucrose phosphorylases) (Jeanes, A., ACS Symp. Ser. 45:284-298 (1977)). Also, many *Leuconostoc* strains are prolific producers of sucrose-inducible extracellular dextranases and are, therefore, abundant producers of dextrans. *Leuconostocs* are used in foods. Finally, they are able to metabolize fructose, which is the byproduct of dextran synthesis, is an energy source. In some instances they can also **produce levans (polyfructoses) by the action of levan sucrase**

US-PAT-NO: 4863719

DOCUMENT-IDENTIFIER: US 4863719 A

TITLE: Microbial production of polyfructose

DATE-ISSUED: September 5, 1989

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------|----------|-------|----------|---------|
| Mays; Thomas D. | Columbia | MD | 21046 | N/A |
| Dally; Ellen L. | Columbia | MD | 21044 | N/A |

APPL-NO: 07/ 232830

DATE FILED: August 16, 1988

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional application of U.S. patent application Ser. No. 06/780,595 filed Sept. 26, 1985 and now U.S. Pat. No. 4,769,254, which in turn is a continuation-in-part of U.S. patent application Ser. No. 06/689,238 filed Jan. 4, 1985, the contents of which are incorporated by reference herein.

US-CL-CURRENT: 424/45; 424/49 ; 424/65 ; 514/777

ABSTRACT:

A water-soluble levan having a weight average molecular weight of about 10,000-40 million, preferably about 5-25 million and especially about 10-20 million which stabilizes a bovine serum albumin colloid having index (EAI) of about 3-100 determined according to the formula $EAI = 2T/OC$ wherein T is turbidity measured at 500 nm, C is the weight of emulsified protein per unit volume of the aqueous phase, and O is the volume fraction of a dispersed oil phase is produced by fermenting a nutrient growth medium feedstock having a carbon source consisting essentially of an assimilable sugar selected from the group consisting of sucrose, raffinose, or a mixture thereof with a microorganism capable of converting at least 50% of the fructose value of the sugar to said levan under nutrient growth conditions which enhance levan production while suppressing ethanol production, It is useful as a colloid stabilizing agent, particularly with foods, beverages, pharmaceuticals, dentifrices, and cosmetics.

15 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

Brief Summary Text - BSTX:

Levan has been used as an immunologic suppressor (Experientia 34: 1362-1363, 1978 and J. Pathol. 125: 103, 1978) and an immunogenic hapten conjugate (J. Dent. Res. 55: 129, 1976 and Moreno, U.S. Pat. No. 4,260,602). UK Patent Application GB 2,046,757-A published Nov. 19, 1980 describes a low cariogenic food sweetener prepared by subjecting a substrate solution containing aldose and sucrose or raffinose to the activity of **levan sucrase, but the desired product is a trisaccharide and not a high molecular weight levan, which has not heretofore been to provide functional properties to food products.**

Brief Summary Text - BSTX:

Traditionally, small quantities of **levan have been produced** by the bacterial fermentation of sucrose, usually by a strain of Actinomyces viscosus or Aerobacter levanicum. **Levan produced by the in vitro activity of levan sucrase** has been reported by T. Tanaka et al. in J. Biochem. 87 (1): 297-303 (1980). The applications of recombinant DNA technology have been applied in the in vitro genetic transfer of the sacB gene from Bacillus subtilis into a strain of Escherichia coli (J. Bacteriol. 153: 1424-31, 1983.) These processes, however, do not represent efficient means for the **production of levan**. In particular, the in vitro enzymatic **synthesis of levan from sucrose would appear to be difficult in terms of producing** a high molecular weight molecule, as evidenced by UK Patent Application GB 2,046,757-A.

Detailed Description Text - DETX:

The applications described herein are based upon the use of a molecule with a weight average molecular weight of 10 to 20 million, as determined by gel filtration chromatography using Bio-Rad "Biogel" agarose having a molecular weight exclusion limit of 15 million using bacterial cells as void markers. It would be difficult to produce such a large molecular weight species through column chromatography of sucrose by an immobilized levan **sucrase** enzyme. The traditional bacterial fermentation of sucrose by strains of Aerobacter levanicum results in only 1.3 to 1.5 grams of levan per 100 milliliters of fermentation broth of the final **levan product** (Methods in Enzymology 8: 161-165, 1966). **Production of levan** by the bacterial fermentation of 2% sucrose by wildtype (or genetically unaltered) strains of Zymomonas results in only 0.2 to 0.3 grams of **levan product** per 100 milliliters of fermentation broth (Biochem. J. 98:804-812, 1966). This represents the utilization of only 2% of the total sucrose substrate present. However, from Table 1, one can see that an efficient wildtype strain in 15% sucrose produces 2.4 grams/100 ml compared with genetically modified and selected progeny which produce 3.4, 2.9 and 2.9 grams/100 ml respectively.

US-PAT-NO: 4855149

DOCUMENT-IDENTIFIER: US 4855149 A

TITLE: Method for producing novel dried compositions containing polysaccharides

DATE-ISSUED: August 8, 1989

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------------|-----------|-------|----------|---------|
| Pucci; Michael J. | Sarasota | FL | N/A | N/A |
| Kunka; Blair S. | Bradenton | FL | N/A | N/A |

APPL-NO: 07/ 068435

DATE FILED: July 1, 1987

US-CL-CURRENT: 426/48; 426/531 ; 426/654 ; 426/657 ; 426/658 ; 435/101

ABSTRACT:

Novel dried compositions containing polysaccharides (dextrans and levans) derived using a *Leuconostoc* to ferment sucrose to produce the polysaccharides are described. In particular, dried compositions incorporating milk solids or other drying aids to facilitate drying and rehydration of the polysaccharides are described. The compositions are useful as quality (e.g. texture, stability or thickness) improvers for foods.

19 Claims, 0 Drawing figures

Exemplary Claim Number: 11

----- KWIC -----

Brief Summary Text - BSTX:

Dextrans are glucose polymers synthesized by several genera of bacteria including *Streptococcus*, *Lactobacillus*, and *Leuconostoc* (Schwartz, R. D., and E. A. Bodie, Appl. Environ. Microbiol. 48:678-679 (1984); and Lawford, G. R., A. Kligerman, and T. Williams, Biotechnol. Bioeng. 21:1121-1131 (1979)). They are generally referred to as "microbial polysaccharides" (Kirk-Othmer 15:439-447 (1981)). Synthesis occurs via dextran sucrose enzymes which are primarily extracellular. Dextran molecular weights range from 1.5.times.10.sup.4 to 2.times.10.sup.7 and higher (Schwartz, R. D., and E. A. Bodie, Appl. Environ. Microbiol. 48:678-679 (1984)). Dextrans consist of primarily alpha 1,6 linkages but alpha 1,4, alpha 1,2 and alpha 1,3 bonds are also known (Niinobe, M. and T. Kobayashi, Nippon Nogeikagaku Kaishi 46:81-88

(1972)). The extent of branching affects water solubility and other properties. Dextran are presently used in a variety of industries; however, although the potential exists, uses of dextran in the food industry are limited at present because of the difficulty in providing a useful dried product which rehydrates in water to produce a suitably thick solution (Jeanes, A., ACS Symp. Ser. 45:284-298 (1977)).

Brief Summary Text - BSTX:

Dextran synthesis has probably been most widely studied in the genera *Leuconostoc*, particularly in *L. mesenteroides* (Lawford, G. R., A. Kligerman, and T. Williams, *Biotechnol. Bioeng.* 21:1121-1131 (1979); Niinobe, M. and T. Kobayashi, *Nippon Nogeikagaku Kaishi* 46:81-88 (1972); and Preobrazhenskaya, M. E. and N. A. Danilova., *Prikladnaya Biokhimiya i Mikrobiologiya* 10:539-546 (1974)). *Leuconostoc* sp. offer several advantages. First, they are unable to metabolize either dextran (they contain no dextranases) or sucrose (no invertases or sucrose phosphorylases) (Jeanes, A. ACS Symp. Ser. 45:284-298 (1977)). Also, many *Leuconostoc* strains are prolific producers of sucrose-inducible extracellular dextranases and are, therefore, abundant producers of dextran. *Leuconostocs* are used in foods. Finally, they are able to metabolize fructose, which is the byproduct of dextran synthesis, as an energy source. In some instances they can also produce levans (polyfructoses) by the action of levan sucrase.

US-PAT-NO: 4769254

DOCUMENT-IDENTIFIER: US 4769254 A

TITLE: Microbial production of polyfructose

DATE-ISSUED: September 6, 1988

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------|----------|-------|----------|---------|
| Mays; Thomas D. | Columbia | MD | N/A | N/A |
| Dally; Ellen L. | Columbia | MD | N/A | N/A |

APPL-NO: 07/ 780595

DATE FILED: September 26, 1985

PARENT-CASE:

DESCRIPTION OF THE INVENTION This application is a continuation-in-part of U.S. patent application Ser. No. 06/689,238 filed Jan. 4, 1985 the contents of which are incorporated by reference herein.

US-CL-CURRENT: 426/564; 426/567 ; 426/568 ; 426/569 ; 426/570 ; 426/571 ; 426/589 ; 426/590 ; 426/605 ; 426/613 ; 426/641 ; 426/643 ; 426/652 ; 426/654 ; 426/658

ABSTRACT:

A water-soluble levan having a weight average molecular weight of about 10,000-40 million, preferably about 5-25 million and especially about 10-20 million which stabilizes a bovine serum albumin colloid having index (EAI) of about 3-100 determined according to the formula $EAI = 2T/OC$ wherein T is turbidity measured at 500 nm, C is the weight of emulsified protein per unit volume of the aqueous phase, and O is the volume fraction of a dispersed oil phase is produced by fermenting a nutrient growth medium feedstock having a carbon source consisting essentially of an assimilable sugar selected from the group consisting of sucrose, raffinose, or a mixture thereof with a microorganism capable of converting at least 50% of the fructose value of the sugar to said levan under nutrient growth conditions which enhance levan production while suppressing ethanol production. It is useful as a colloid stabilizing agent, particularly with foods, beverages, pharmaceuticals, dentifrices, and cosmetics.

13 Claims, 4 Drawing figures

Exemplary Claim Number: 13

Number of Drawing Sheets: 4

----- KWIC -----

Brief Summary Text - BSTX:

This invention relates to a water-soluble, essentially uncharged levan polymer which has useful emulsifying and thickening properties comparable to those of gum arabic, and to methods for the production and use thereof as well as compositions containing said levan polymer as an emulsifying agent. More particularly, this invention relates to the production of levan through the action of levan sucrase produced by an improved and modified bacterial strain of *Zymomonas mobilis*.

Brief Summary Text - BSTX:

Levan has been used as an immunologic suppressor (*Experientia* 34: 1362-1363, 1978 and *J. Pathol.* 125: 103, 1978) and an immunogenic hapten conjugate (*J. Dent. Res.* 55: 129, 1976 and Moreno, U.S. Pat. No. 4,260,602). UK patent Application GB No. 2,046,757-A published Nov. 19, 1980 describes a low cariogenic food sweetener prepared by subjecting a substrate solution containing aldose and sucrose or raffinose to the activity of levan sucrase, but the desired product is a trisaccharide and not a high molecular weight levan, which has not heretofore been to provide functional properties to food products.

Brief Summary Text - BSTX:

Traditionally, small quantities of levan have been produced by the bacterial fermentation of sucrose, usually by a strain of *Actinomyces viscosus* or *Aerobacter levanicum*. Levan produced by the in vitro activity of levan sucrase has been reported by T. Tanaka et al. in *J. Biochem.* 87 (1): 297-303 (1980). The applications of recombinant DNA technology have been applied in the in vitro genetic transfer of the *sacB* gene from *Bacillus subtilis* into a strain of *Escherichia coli* (*J. Bacteriol.* 153: 1424-31, 1983). These processes, however, do not represent efficient means for the production of levan. In particular, the in vitro enzymatic synthesis of levan from sucrose would appear to be difficult in terms of producing a high molecular weight molecule, as evidenced by UK Patent Application GB No. 2,046,757-A.

Detailed Description Text - DETX:

The applications described herein are based upon the use of a molecule with a weight average molecular weight of 10 to 20 million, as determined by gel filtration chromatography using Bio-Rad "Biogel" agarose having a molecular weight exclusion limit of 15 million using bacterial cells as void markers. It would be difficult to produce such a large molecular weight species through column chromatography of sucrose by an immobilized levan sucrase enzyme. The traditional bacterial fermentation of sucrose by strains of *Aerobacter*

levanicum results in only 1.3 to 1.5 grams of levan per 100 milliliters of fermentation broth of the final levan pr duct (Methods in Enzymology 8: 161-165, 1966). Pr duction f levan by the bacterial fermentation of 2% sucrose by wildtype (or genetically unaltered) strains of Zymomonas results in only 0.2 to 0.3 grams of levan product per 100 milliliters of fermentation broth (Biochem. J. 98 :804-812, 1966). This represents the utilization of only 2% of the total sucrose substrate present. However, from Table 1, one can see that an efficient wildtype strain in 15% sucrose produces 2.4 grams/100 ml compared with genetically modified and selected progeny which produce 3.4, 2.9 and 2.9 grams/100 ml respectively.

US-PAT-NO: 4749695

DOCUMENT-IDENTIFIER: US 4749695 A

TITLE: Water soluble iron dextran and a process for its manufacture

DATE-ISSUED: June 7, 1988

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|----------|-------|----------|---------|
| Schwengers; Dieter | Dormagen | N/A | N/A | DE |

APPL-NO: 06/ 741395

DATE FILED: June 5, 1985

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| DE | 3422249 | June 15, 1984 |

US-CL-CURRENT: 514/59; 536/112 ; 536/113

ABSTRACT:

A water-soluble iron dextran having a high iron content is prepared by adding to an aqueous solution containing more than 200 mmol D-glucose per 1000 U.alpha.(1.fwdarw.6)-D- glucosyltransferase, at 265 to 310K and a pH value of 4.5 to 8, an aqueous sucrose solution in a mole ratio of sucrose to glucose of from 2.0 to 5.0; separating, after the consumption of the sucrose, glucose, liberated fructose and undesired oligosaccharides; reacting the so-purified dextran, having an average molar mass of from 2000 to 4000, with freshly precipitated iron (III) hydroxide and, if desired, further purifying the same.

Iron dextrans having an iron content of from 27 to 33 percent by weight and an average molar mass of the dextran component of from 2000 to 4000, may be prepared according to the process of the invention which can be used in the treatment of iron deficiency.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1,2

----- KWIC -----

Brief Summary Text - BSTX:

According to the classification of the "Enzyme Commission", enzymes which transfer the D-glucopyranosyl group of sucrose to suitable acceptors are

designated as α -(1 \rightarrow 6)-D-glucosyl transferase. An extracellular enzyme of the kind is dextran sucrase (E.C. 2.4.1.5) which is formed by specific kinds of bacteria of the lactobacilli species, for example, *Leuconostoc mesenteroides*, in particular the strain B-512, *Leuconostoc dextranicum*, *Streptococcus* and *lactobacillus*. When preparing native dextran, sucrose serves primarily as acceptor and acts as chain initiator for a chain polymerization in which by virtue of continuous transfer of D-glucopyranosyl groups from the sucrose to the growing chain of the polysaccharide, dextrans having molar masses of several millions are formed, while, at the same time, a fructose molecule is liberated for each reacted molecule of sucrose.

US-PAT-NO: 4742006

DOCUMENT-IDENTIFIER: US 4742006 A

TITLE: Fermentation process for the production of fructose from aqueous mixtures of fructose and glucose and Zymomonas mobilis mutants which can be used for such fermentation

DATE-ISSUED: May 3, 1988

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|--------|-------|----------|---------|
| Bringer, Stephanie | Julich | N/A | N/A | DE |
| Sahm; Hermann | Julich | N/A | N/A | DE |

APPL-NO: 06/ 795422

DATE FILED: November 6, 1985

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|-----------------|
| DE | 3528933 | August 13, 1985 |

US-CL-CURRENT: 435/105; 435/252.1 ; 435/274 ; 435/822

ABSTRACT:

A method of producing fructose from aqueous glucose and fructose mixtures by fermenting in one step an aqueous mixture of fructose and glucose with a stable mutant of Zymomonas mobilis, and also producing the stable mutant of Zymomonas mobilis.

14 Claims, 0 Drawing figures

Exemplary Claim Number: 11

----- KWIC -----

Brief Summary Text - BSTX:

In Australian Patent Abstract No. AU-A 29 530/84, which is incorporated herein by reference, a process is described for the production of fructose and ethanol from sucrose, where the sucrose concentration must be greater than or equal to 30 percent by weight. By means of the bacterium Zymomonas mobilis, preferably after pre-treatment of the sucrose solution with an immobilized levan sucrase enzyme preparation (EC 2.4.1.10), there is a selective conversion of the glucose to ethanol within 1-2 days. However, this process also has significant disadvantages, in that the enzyme levan sucrase, according to EC 2.4.1.10, is

in effect a fructosyltransferase, that is, the reaction products from sucrose are polyfructose (levan) and glucose. (T. E. Barman, 1969, Enzyme Handbook I, page 310, which is incorporated herein by reference.) It is also known that Zymomonas also produces levan from sucrose (D. W. Ribbons, 1962, Biochem. J. 82, 45 p; E. A. Dawes et al., 1966, Biochem. J. 98, 804; K. J. Lee, 1981, Biotechnol. Lett. 3 207, incorporated herein by reference.) The process according to the Australian Patent AU-A 29 530/84 also leads to the formation of levan, and therefore to significant slime formation. Naturally, the formation of levan also causes losses of monomer fructose. In addition, known strains of Zymomonas mobilis also convert fructose to ethanol, which also necessarily decreases the fructose yield in the above process. (Swings and DeLey, 1977, Bacteriol. Rev. 41, 1, incorporated herein by reference.)

US-PAT-NO: 4673643

DOCUMENT-IDENTIFIER: US 4673643 A

TITLE: Process for the manufacture of iso-malto-oligosaccharide monovalent haptens

DATE-ISSUED: June 16, 1987

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|----------|-------|----------|---------|
| Schwengers; Dieter | Dormagen | N/A | N/A | DE |

APPL-NO: 06/ 743715

DATE FILED: June 11, 1985

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| DE | 3422246 | June 15, 1984 |

US-CL-CURRENT: 435/97; 435/103

ABSTRACT:

Iso-malto-oligosaccharide monovalent haptens were prepared by adding an aqueous sucrose solution to an aqueous solution of D-glucose containing more than 300 mmol glucose per 1000 U .alpha.(1.fwdarw.6)-D-glucosyl transferase at 265 to 310 K and a pH value of from 4.5 to 8.0 and a molar ratio of sucrose to glucose of from 0.5 to 2.0. After consumption of the sucrose, glucose, liberated fructose and undesired oligosaccharides are separated in a known manner.

The process of the invention allows a particularly economical preparation of the monovalent haptens which serve for the prophylaxis of undesired dextran induced anaphylactoid side effects (DIAR).

16 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

According to the classification of the "Enzyme Commission", enzymes which transfer the D-glucopyranosyl group of sucrose to suitable acceptors are designated as .alpha.(1.fwdarw.6)-D-glucosyl transferase. An extracellular

enzyme of this kind is dextran sucrase (E.C. 2.4.1.5) which is formed by specific kinds of bacteria of the lactobacilli species, for example, *Leuconostoc mesenteroides*, in particular the strain B-512, *Leuconostoc dextranicum*, *Streptococcus* and *Lactobacillus*. When preparing dextran, sucrose serves primarily as acceptor and acts as chain initiator for a chain polymerization in which by virtue of continuous transfer of D-glucopyranosyl groups from the sucrose to the growing chain the polysaccharide dextran having molecular masses of several millions is formed, while, at the same time, a fructose molecule is liberated for each reacted molecule of sucrose.

US-PAT-NO: 4649058

DOCUMENT-IDENTIFIER: US 4649058 A

TITLE: Gluco-oligosaccharide mixture and a process for its manufacture

DATE-ISSUED: March 10, 1987

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|----------|-------|----------|---------|
| Schwengers; Dieter | Dormagen | N/A | N/A | DE |

APPL-NO: 06/ 741394

DATE FILED: June 5, 1985

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| DE | 3422247 | June 15, 1984 |

US-CL-CURRENT: 426/658; 426/804 ; 435/78 ; 435/96 ; 435/97 ; 435/99

ABSTRACT:

A gluco-oligosaccharide mixture having up to 30, in particular from 10 to 20, anhydroglucose units is prepared by reacting an aqueous solution of a mono-or disaccharide composed of glucose units containing more than 200 mmol of the saccharide per 1000 U .alpha.(1.fwdarw.6)-D-glucosyl transferase, at 265 to 310 K and a pH value of from 4.5 to 8.0, with an aqueous solution of sucrose in a molar ratio of sucrose to glucose of 2.0 to 5.0.

The oligosaccharide mixtures of the present invention are used as calorie-free carrier for sweetening agents and as "body building" sweetening agent.

18 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

According to the classification of the "Enzyme Commission", enzymes which transfer the D-glucopyranosyl group of sucrose to suitable acceptors are designated as .alpha. (1.fwdarw.6)-D-glucosyl transferase. An extracellular enzyme of the kind is dextran sucras (E.C. 2.4.1.5). which is formed by specific kinds of bacteria of the lactobacilli species, for example,

Leuconostoc mesenteroides, in particular the strain B-512, Leuconostoc dextranicum, Streptococcus and **lact bacillus**. When preparing dextran, sucrose serves primarily as acceptor and acts as chain initiator for a chain polymerization in which by virtue of continuous transfer of D-glucopyranosyl groups from the sucrose to the growing chain of the polysaccharide, dextrans having molecular masses of several millions are formed, while, at the same time, a fructose molecule is liberated for each reacted molecule of sucrose.

US-PAT-NO: 4617269

DOCUMENT-IDENTIFIER: US 4617269 A

TITLE: Process for the preparation of fructosyl disaccharides

DATE-ISSUED: October 14, 1986

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------|-----------|-------|----------|---------|
| Rathbone; Elner B. | Wokingham | N/A | N/A | GB2 |
| Hacking; Andrew J. | Reading | N/A | N/A | GB2 |
| Cheetham; Peter S. J. | Reading | N/A | N/A | GB2 |

APPL-NO: 06/ 622853

DATE FILED: June 21, 1984

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|---------|---------------|
| GB | 8316790 | June 21, 1983 |

US-CL-CURRENT: 435/97; 435/193 ; 435/839

ABSTRACT:

A process for preparing a fructoside, especially a fructosyl disaccharide, comprises reacting a fructosyl saccharide such as sucrose or raffinose with an alcohol or aldose in the presence of a fructosyl-transferase, especially one derived from *B. subtilis* NCIB 11811, 11872 or 11873. In particular, aldose is a compound of the formula ##STR1## in which A represents a hydrogen atom or the group CH.sub.2 X, where X represents a hydrogen atom or an alkoxy group, and the fructosyl disaccharide so formed is halogenated to provide a halosucrose or halogalactosucrose sweetener.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 7

----- KWIC -----

Brief Summary Text - BSTX:

The enzyme in question is a fructosyltransferase. Fructosyltransferases are well known in enzymology. A representative enzyme is the so-called levansucrase, responsible for the production of levan, a polyfructose derivative, in the decomposition of sucrose or of raffinose. In its normal mode of action, levansucrase splits the glucose-fructose link in sucrose and

transfers the fructose to an acceptor sugar, e.g. sucrose itself. This process is repeated so that fructose chains are built up. If another sugar is present besides sucrose, e.g. D-xylose, the levan formation is inhibited, or at least reduced, and instead the fructose is transferred to the other competing sugar which acts as an acceptor to produce a new fructoside. The new fructoside will also act as a donor, so in practice a large excess of donor has been used in order to push the equilibrium in the desired direction.

Brief Summary Text - BSTX:

We have now found that 6-derivatised sucrose derivatives can be prepared by reacting the corresponding 6-derivatised glucose or galactose with a **fructosyl transferase** in the presence of sucrose or raffinose or stachyose. The product can then be halogenated in the 4,1' and 6'-positions and, if desired, the 6-derivatising group removed to yield the required halosugar. The initial reaction proceeds in good yield in the absence of the **production of any levan**.

Brief Summary Text - BSTX:

The **fructosyltransferase** derived from many strains of B.subtilis and Erwinia sp are generally regarded as being levansucrases: that is to say that in the presence of sucrose, they cause the **production of levan**, a polyfructose material which is alcohol precipitable. When they are used in the production of fructose disaccharides, the competing reaction to **produce levan must be suppressed if any useful product** is to be obtained, hence the restriction of these enzymes in GB No. 2046757A to reaction mixture containing high proportions of the acceptor molecule. However the B.subtilis NCIB 11871, 11872 and 11873 enzymes used here are much less prone to **produce levan**. The K.sub.m for sucrose for '**levan**' **production** is about 0.2M. This compares with a quoted K.sub.m of about 0.02M for the Dedonder (loc.cit.) BS5 strain enzyme. Even when equivalent concentrations of the acceptor and donor molecules are used and when the conditions are used which were found to promote the **synthesis of high molecular weight levan** by the Tanaka B.subtilis enzyme (i.e. addition of levan primer, use of a low ionic strength solution, and reaction at low temperatures (J.Biochem 90, 521, 1981) very little high molecular weight **levan is produced**. Only after the peak yield of disaccharide is reached is a polymer of intermediate molecular weight formed. Furthermore, unlike other true levansucrases, the enzyme from B.subtilis NCIB 11871 appears not to catalyse a disproportionation reaction i.e. it does not convert low molecular weight oligosaccharides into high molecular weight levan. For instance trisaccharide can be detected, which should not be present if the enzyme carries out the disproportionation reaction. Standard levan obtained from Aerobacter levanicum (Sigma) can be fractionated into two peaks corresponding to high and intermediate molecular weight material. The Dedonder (loc.cit.) enzyme has an equilibrium constant (levan and glucose/sucrose) of about 3.6.times.10.sup.-2 at 37.degree. C., levan of DP40 being formed. In complete contrast, strains NCIB 11871, 11872 and 11873 produce an enzyme which produces no significant amount of alcohol-precipitable polysaccharide from sucrose alone, and even the growing cells of strain 11871 **produce no levan**. It thus appears that the **fruct syltransferase produced is not effectively a 'levan sucrose' at all**. In

this specification it will be referred to as a fructosyltransferase.

US-PAT-NO: 4591563

DOCUMENT-IDENTIFIER: US 4591563 A

TITLE: Process for the purification of dextran-sucrase

DATE-ISSUED: May 27, 1986

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------|-------------|-------|----------|---------|
| Paul; Francois | Saint-Orens | N/A | N/A | FR |
| Monsan; Pierre | Blagnac | N/A | N/A | FR |
| Auriol; Daniel | Maury | N/A | N/A | FR |

APPL-NO: 06/ 606642

DATE FILED: May 3, 1984

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-------------|
| FR | 83 07650 | May 6, 1983 |

US-CL-CURRENT: 435/193; 435/814 ; 435/816 ; 435/822

ABSTRACT:

The invention is directed to a process for the purification of the dextran-sucrase produced by *Leuconostoc mesenteroides* bacteria.

The process according to the invention consists of adding to the culture medium, that contains the extra-cellular enzyme and dextran, a quantity of a PEG type polyether so that, in the medium appear two non miscible phases, that are thus maintained under stirring in order to obtain a good contact. Thereafter the lower dextran phase is separated from the upper polyether phase in order to provide a dextran-sucrase enriched enzymatic preparation.

The purified enzyme can be used in the synthesis processes of the dextrans possessing specific molecular weights for certain applications.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Numerous bacterial layers forming part of the Lactobacillus, *Streptococcus* or

Leuconostoc species possess a **dextran-sucrase** activity. Among these, however, the strain of Leuconostoc mesenteroides NRRL B 512 (F), that excretes the **dextran-sucrase** enzyme, when it is cultivated in the presence of sucrose, presents particularly interesting stability properties, an important productivity and a non-pathogenic character, that have allowed it to be utilized on an industrial scale. Furthermore, the **dextran-sucrase** obtained from this bacterial strain produces an only slightly branched linear dextran (95% α -1,6 glycosidic bonds) which is soluble in water, qualities which render it a very worthwhile product on the industrial scale.

Brief Summary Text - BSTX:

If a glucose production is observed during measurement of the saccharolytic activity, it results from a contaminating activity, invertase or **levan-sucrase**. However, with the aim of allowing the results to be clearly expressed, the **production of glucose has been attributed to a levan-sucrase** activity that is largely preponderant. Therefore, the levan-sucrase activity and the **dextran-sucrase** activity that appear in the tables are determined as follows:

US-PAT-NO: 4444793

DOCUMENT-IDENTIFIER: US 4444793 A

TITLE: Fermentation of whey to produce a thickening agent

DATE-ISSUED: April 24, 1984

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|------------|-------|----------|---------|
| Schwartz; Robert D. | Concord | CA | N/A | N/A |
| Bodie; Elizabeth A. | El Cerrito | CA | N/A | N/A |

DISCLAIMER DATE: 20000816

APPL-NO: 06/ 290766

DATE FILED: August 7, 1981

US-CL-CURRENT: 426/41; 426/43 ; 435/101 ; 435/103 ; 435/822

ABSTRACT:

Dairy whey, a waste product of cheese production, is fermented to produce a thickening agent for use in the food industry by forming a fermentation broth containing whey and sucrose, and fermenting the broth with *Leuconostoc mesenteroides* ATCC 14935. The fermentation broth can optionally contain a water-soluble phosphate as a pH buffer and yeast extract.

9 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 3

----- KWIC -----

Detailed Description Text - DETX:

Dextrans are polyglucans that are synthesized from sucrose by many species of the genera *Leuconostoc*, *Lactobacillus*, and *Streptococcus*. The synthesis occurs extracellularly and is catalyzed by a species specific enzyme, dextran sucrose. Soluble and insoluble dextrans are produced and molecular weights range from 1.5.times.10.sup.4 -2.times.10.sup.7 and higher. Although dextran has the potential to be used in food products as a conditioner, stabilizer, "bodying agent," etc., it has not found wide commercial use in the food industry. Dextran has been used in gel filtration processes to concentrate proteins or to recover proteins from liquid wastes, including cheese whey fractionation.

Dextran solutions are reported to have properties similar to locust bean gum; see, Brooker, 1979, "Electron Microscopy of Dextrans Produced by Lactic Acid Bacteria," Microbial Polysaccharides and Polysaccharases, Berkeley et al. Eds, pp. 84-115, Academic Press, N.Y.; Jeans, 1977, "Dextrans and Pullulans: Industrially Significant D-glucans," Extracellular Microbial Polysaccharides, Sanford et al. Eds., pp. 284-298, ACS, Washington, D.C.; Kang et al., 1979, "Polysaccharides," Microbial Technology, 2nd Ed., Vol. 1. pp. 417-481, Academic Press, N.Y.; Lawford et al., 1979, "Dextran Biosynthesis and Dextranase Production by Continuous Culture of *Leuconostoc mesenteroides*," Biotech. Bioeng. 21:1121-1131; and Wells, 1977, "Extracellular Microbial Polysaccharides--A Critical Overview," Extracellular Microbial Polysaccharides, ACS Symposium Series 45, pp. 299-313, ACS, Washington, D.C.

US-PAT-NO: 4396602

DOCUMENT-IDENTIFIER: US 4396602 A

TITLE: Blood glucose level lowering agents

DATE-ISSUED: August 2, 1983

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-------------|-------|-------|----------|---------|
| Endo; Akira | Tokyo | N/A | N/A | JP |

APPL-NO: 06/ 304467

DATE FILED: September 22, 1981

US-CL-CURRENT: 424/94.5

ABSTRACT:

A method of lowering the blood glucose level in mammals and a blood glucose level-lowering agent are described. The method comprises administering an enzyme capable of synthesizing sparingly-digestible saccharides from easily-digestible saccharides. The blood glucose level-lowering agent comprises the enzyme capable of synthesizing sparingly-digestible saccharides from easily-digestible saccharides and a glucosidase-inhibiting agent.

12 Claims, 0 Drawing figures

Exemplary Claim Number: 1,2

----- KWIC -----

Brief Summary Text - BSTX:

Dextranase, produced by the genera Leuconostoc, Streptococcus, and Betabacterium, which synthesizes dextran from sucrose; Dextran 6-glycosyltransferase, produced by the genus Acetobacter, which synthesizes dextran from amylopectin; Levansucrase, produced by the genera Bacillus, Acetobacter, Pseudomonas, and Xanthomonas, which synthesizes levan from sucrose; Amylomaltase, produced by the genus Escherichia, which synthesizes amylose from maltose; Amylosucrase, produced by the genus Neisseria, which synthesizes amylopectin-like polysaccharide from sucrose; and cyclodextrin-synthesizing enzymes, produced by the genera Bacillus and Klebsiella, which synthesize cyclodextrin (.alpha., .beta., and .GAMMA. types) from starch or sucrose. Of these enzymes, dextranase and cyclodextrin-synthesizing enzymes are particularly preferred. These enzymes are all known and can be prepared by known procedures (e.g., W. Pigman and D.

Horton, Ed., *The Carbohydrates. Chemistry and Biochemistry*, Academic Press, pp. 302-373 (1970); S. P. Colowick and N. O. Kaplan, Ed., *Methods in Enzymology*, Academic Press, Vol. 1, pp. 178-192 (1955), and Vol. 5, pp. 140-155 (1962); E. F. Neufeld and V. Ginsberg, Ed., *Methods in Enzymology*, Vol. 8, pp. 500-505 (1966); T. J. Montville et al., *Advances in Applied Microbiology*, Academic Press, Vol. 24, pp. 55-84 (1978); H. Horikoshi and N. Nakamura, *Kagaku to Seibutsu*, Vol. 17, pp. 300-305 (1979); K. Matsuda and M. Kobayashi, *Hakko to Kogyo*, Vol. 36, pp. 11-21 (1978); S. Kobayashi and K. Kainuma, *Hakko to Kogyo*, Vol. 36, pp. 176-183 (1978); and T. Tanaka, S. Oi, M. Iizuka, and T. Yamamoto, *Agric. Biol. Chem.*, Vol. 42, pp. 323-326 (1978)).

US-PAT-NO: 4335100

DOCUMENT-IDENTIFIER: US 4335100 A

TITLE: Method of inhibiting dextran sucrose and oral compositions for use therein

DATE-ISSUED: June 15, 1982

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|---------|-------|----------|---------|
| Robyt; John F. | Ames | IA | N/A | N/A |
| Zikopoulos; John N. | Phoenix | AZ | N/A | N/A |

APPL-NO: 06/ 175635

DATE FILED: August 6, 1980

PARENT-CASE:

CROSS-REFERENCE This application is a continuation-in-part of co-pending application Ser. No. 32,398, filed Apr. 23, 1979, now U.S. Pat. No. 4,228,150.

US-CL-CURRENT: 424/48; 424/49 ; 426/3 ; 426/660 ; 514/23 ; 536/1.11 ; 536/122

ABSTRACT:

Dextran sucrose synthesis of dextran from sucrose is inhibited by substituted sucrose compounds which have an inhibiting group bonded to the 5-position ring carbon. Oral compositions containing such substituted sucrose compounds can be used to control dextran formation in the mouth, and dextran comprises the principal component of dental plaque.

14 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

The method of the present invention has utility for inhibiting the dextran sucrose synthesis of dextran from sucrose. By introducing the substituted sucrose into the oral cavity the enzymes can be effectively inhibited. By this procedure, the biosynthesis of dextran can be controlled or prevented. Thus, by combining the substituted sucrose with a carrier suitable for use in the oral cavity, such as toothpaste, mouthwash, or chewing gum, an

oral vehicle is provided for the control of plaque formation in the mouth. With certain of the substituted sucroses, the levansucrase synthesis of levan from sucrose is also inhibited. This is believed to be desirable in minimizing the formation of dental plaque, which also contain levan, although in relatively small amounts compared to its dextran content.

US-PAT-NO: 4250262

DOCUMENT-IDENTIFIER: US 4250262 A

TITLE: Method of preparing a purified glucosyltransferase

DATE-ISSUED: February 10, 1981

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|--------|-------|----------|---------|
| Taubman; Martin A. | Newton | MA | N/A | N/A |
| Smith; Daniel J. | Natick | MA | N/A | N/A |

APPL-NO: 06/ 103590

DATE FILED: December 14, 1979

PARENT-CASE:

This is a continuation of application Ser. No. 956,847, filed Nov. 2, 1978, now abandoned (which application is a division of Ser. No. 879,432, filed Feb. 21, 1978 (now U.S. Pat. No. 4,150,116, issued Apr. 17, 1979).

US-CL-CURRENT: 435/193; 435/815 ; 435/885

ABSTRACT:

Immunization of animals with preparations containing more purified forms of glucosyltransferase (GTF) results in the presence of antibody in saliva demonstrable by functional inhibitions of enzyme activity and binding of radioactive enzyme. Serum antibody was also present. Immunized groups of animals had lower mean caries scores than comparably sham-immunized or nonimmunized control groups. Local immunization with GTF of serotype c or g of a Streptococcus mutans reduces the colonization, caries, and lesions caused by infection with S. mutans of serotype g (strain 6715) or c, or with serotype g or c, or with serotype a or g, respectively.

13 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

A number of vaccines have been proposed for immunization against dental caries in animals. Various of these proposals are summarized in U.S. Pat. No. 3,879,545. The invention claimed in that patent relates to caries-preventive

vaccines incorporating as the active ingredient thereof a polyfructan (or levan) polysaccharide produced by elaboration of certain strains of streptococcus, particularly Streptococcus Strain SS2. The immunization technique, according to the patentee, has been found to result in the formation of antibodies against the heterogeneous micro-organisms in the reticuloendothelial system and in the blood, and to result in significant decreases in the formation of carious lesions in host animals subjected to inoculation with such organisms. However, the patentees also disclose investigating the use of dextranucrase enzyme for immunization against caries formation.

Brief Summary Text - BSTX:

As disclosed in U.S. Pat. No. 3,879,545, the patentees had believed that since the enzymes dextranucrase and levansucrase are produced by S. mutans and S. Strain SS2, respectively, and are involved in the synthesis of dextran and levan, and these polysaccharides are believed to produce caries, that immunization with these enzymes might result in the production of antibodies to the enzymes which would neutralize their activity in vivo, thus inhibiting synthesis of the polysaccharides and resulting in decreased plaque formations and lowered incidence of caries. The patentees disclose, however, that their experimentations failed to confirm this hypothesis. Nevertheless, the data disclosed did appear to show that while enzyme preparations purified as described by Guggenheim and Newbrun in Helv. Odontol. Acta. 13:84-97 (1969) were ineffective in two routes of systemic immunization, a more crude form was somewhat effective against infectious inoculations with S. mutans 6715.

US-PAT-NO: 4228150

DOCUMENT-IDENTIFIER: US 4228150 A

TITLE: Method of inhibiting dextran sucrase and oral compositions for use therein

DATE-ISSUED: October 14, 1980

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|------|-------|----------|---------|
| Robyt; John F. | Ames | IA | N/A | N/A |
| Zikopoulos; John N. | Ames | IA | N/A | N/A |

APPL-NO: 06/ 032398

DATE FILED: April 23, 1979

US-CL-CURRENT: 424/48; 127/29 ; 127/30 ; 424/52 ; 426/3 ; 426/6 ; 426/658 ; 426/660 ; 426/74 ; 426/9 ; 435/184 ; 435/193 ; 514/835

ABSTRACT:

Dextran sucrase synthesis of dextran from sucrose is inhibited by novel fluorosucroses which are substituted with fluorine for at least the C.sub.6 hydroxyl, and which may also be substituted with fluorine for other hydroxyls. Oral compositions containing such fluorosucrose can be used to control dextran formation in the mouth. Dextran comprises the principal component of dental plaque.

26 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

The method of the present invention has utility for inhibiting the dextran sucrase synthesis of dextran from sucrose. By introducing the mixture of fluorosucroses into the oral cavity the enzymes can be effectively inhibited. By this procedure, the biosynthesis of dextran can be controlled or prevented. Thus, by combining the fluorosucroses with a carrier suitable for use in the oral cavity, such as toothpaste, mouthwash, or chewing gum, an oral vehicle is provided for the control of plaque formation in the mouth. With certain of the fluorosucroses, the levan sucrase synthesis of levan from sucrose is also inhibited. This is believed to be desirable in minimizing the formation of dental plaque, which also contain levan, although in relatively

small amounts compared to its dextran content.

US-PAT-NO: 4150116

DOCUMENT-IDENTIFIER: US 4150116 A

TITLE: Immunization against dental caries with glucosyltransferase antigens

DATE-ISSUED: April 17, 1979

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|--------|-------|----------|---------|
| Taubman; Martin A. | Newton | MA | N/A | N/A |
| Smith; Daniel J. | Natick | MA | N/A | N/A |

APPL-NO: 05/ 879432

DATE FILED: February 21, 1978

US-CL-CURRENT: 424/244.1; 424/50 ; 424/94.5 ; 435/193

ABSTRACT:

Immunization of animals with preparations containing more purified forms of glucosyltransferase (GTF) results in the presence of antibody in saliva demonstrable by functional inhibitions of enzyme activity and binding of radioactive enzyme. Serum antibody was also present. Immunized groups of animals had lower mean caries scores than comparably sham-immunized or nonimmunized control groups. Local immunization with GTF of serotype c or g or a Streptococcus mutans reduces the colonization, caries, and lesions caused by infection with S. mutans of serotype g (strain 6715) or c, or with serotype g or c, or with serotype a or g, respectively.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

A number of vaccines have been proposed for immunization against dental caries in animals. Various of these proposals are summarized in U.S. Pat. No. 3,879,545. The invention claimed in that patent relates to caries-preventive vaccines incorporating as the active ingredient thereof a polyfructan (or **levan**) **polysaccharide produced** by elaboration of certain strains of streptococcus, particularly Streptococcus Strain SS2. The immunization technique, according to the patentee, has been found to result in the formation of antibodies against the heterogeneous micro-organisms in the reticuloendothelial system and in the blood, and to result in significant

decreases in the formation of caries lesions in host animals subjected to inoculation with such organisms. However, the patentees also disclose investigating the use of dextranucrase enzyme for immunization against caries formation.

Brief Summary Text - BSTX:

As disclosed in U.S. Pat. No. 3,879,545, the patentees had believed that since the enzymes dextranucrase and levansucrase are produced by *S. mutans* and *S. Strain SS2*, respectively, and are involved in the synthesis of dextran and levan, and these polysaccharides are believed to produce caries, that immunization with these enzymes might result in the production of antibodies to the enzymes which would neutralize their activity in vivo, thus inhibiting synthesis of the polysaccharides and resulting in decreased plaque formations and lowered incidence of caries. The patentees disclose, however, that their experimentations failed to confirm this hypothesis. Nevertheless, the data disclosed did appear to show that while enzyme preparations purified as described by Guggenheim and Newbrun in *Helv. Odontol. Acta.* 13:84-97(1969) were ineffective in two routes of systemic immunization, a more crude form was somewhat effective against infectious inoculations with *S. mutans* 6715.

US-PAT-NO: 3993747

DOCUMENT-IDENTIFIER: US 3993747 A

TITLE: Method for local immunization against dental caries

DATE-ISSUED: November 23, 1976

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------------------|------------|-------|----------|---------|
| Gaffar; Abdul | Somerset | NJ | N/A | N/A |
| Marcussen, Jr.; Hans Wilhelm | Piscataway | NJ | N/A | N/A |

APPL-NO: 05/ 614384

DATE FILED: September 18, 1975

PARENT-CASE:

This is a divisional of application Ser. No. 371,842, filed June 20, 1973, allowed as U.S. Pat. No. 3,931,398, issued Jan. 6, 1976.

US-CL-CURRENT: 424/244.1; 424/50 ; 424/804 ; 424/94.5 ; 424/94.61 ; 514/885

ABSTRACT:

Immunization against dental caries by local administration, in the vicinity of the oral mucosa of an animal susceptible to dental caries, of a vaccine containing a polyfructan or polyglucan polysaccharide, or a levansucrase or dextransucrase enzyme. The polysaccharides may be administered in purified form, or may be in the form of dead cells of the streptococci by which they are elaborated. Polyglucan polysaccharides so useful are produced by elaboration of strains of the species *S.mutans* and *S.sanguis*, while the polyfructan which may be incorporated in the vaccine may be an elaboration product of *Streptomyces* Strain SS2.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

As indicated hereinabove, animals susceptible to dental caries are immunized, utilizing the present technique, by local administration of a vaccine in the vicinity of the oral mucosa of the animal. The vaccines locally administered

by the method hereof may comprise, as the active ingredient thereof, a polysaccharide elaboration product of a cariogenic Streptococcus organism, i.e., a polyglucan elaboration product of a strain of S.mutans or a strain of S.sanguis; or a polyfructan **(levan) elaboration product** of, for example, Streptococcus Strain SS2. Alternatively, it has been found that the enzymes involved in the synthesis of the noted polysaccharides, i.e., **dextransucrase and levansucrase**, are effective immunizing agents against caries when administered locally in the vicinity of the oral mucosa in accordance with the present invention.

US-PAT-NO: 3952092

DOCUMENT-IDENTIFIER: US 3952092 A

TITLE: Oral preparations

DATE-ISSUED: April 20, 1976

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|--------------------------|-------|----------|---------|
| Bowen; William Henry | Purley, Surrey | N/A | N/A | EN |
| Barker; Sidney Alan | Selly Oak, Birmingham | N/A | N/A | EN |

APPL-NO: 05/ 367892

DATE FILED: June 7, 1973

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|--------------|
| UK | 27048/72 | June 9, 1972 |

US-CL-CURRENT: 424/50; 424/52

ABSTRACT:

The present invention provides a composition which comprises at least one agglutinin which is a site-directed homing agent attached to a biologically active molecule which is effective in modifying the nature of a target site. There is also provided a process for the production of a composition which comprises attaching at least one agglutinin which is a site-directed homing agent to a biologically active molecule which is effective in modifying the nature of a target site.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

Whilst the present invention is particularly described with reference to the use of dextranase as the biologically active molecule, it is not restricted thereto. The biologically active molecule may be, for example, an enzyme catalyst an antibiotic, a protein complexed with fluoride, any other antiplaque or anti-caries molecule or any combination thereof. Other enzymes which may be used include levanase, enzymes that can assist in the oral digestion of food

such as .alpha.-amylase and glucamylase which act on starch and dextrin components of food, lysozyme (muramidase) which is known to be present in human saliva, and substances of the anti-**lact bacillus** system (as described by Bowen, W. H. on page 487 of *Biology of the Periodontium* edited by A. H. Melcher and W. H. Bowen, Academic Press, 1969). Such enzymes can be used singly or in combination with one another. This is particularly true of mixtures of dextranase and amylase. Other anti-plaque or anti-caries substances which may be used include fluorides, calcium glycerophosphate, antibiotics and dextran **sucrase** inhibitors.

US-PAT-NO: 3931398

DOCUMENT-IDENTIFIER: US 3931398 A

TITLE: Method for local immunization against dental caries

DATE-ISSUED: January 6, 1976

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------------------|------------|-------|----------|---------|
| Gaffar; Abdul | Somerset | NJ | N/A | N/A |
| Marcussen, Jr.; Hans Wilhelm | Piscataway | NJ | N/A | N/A |

APPL-NO: 05/ 371842

DATE FILED: June 20, 1973

US-CL-CURRENT: 424/244.1; 424/50 ; 435/885

ABSTRACT:

Immunization against dental caries by local administration, in the vicinity of the oral mucosa of an animal susceptible to dental caries, of a vaccine containing a polyfructan or polyglucan polysaccharide, or a levansucrase or dextranucrase enzyme. The polysaccharides may be administered in purified form, or may be in the form of dead cells of the streptococci by which they are elaborated. Polyglucan polysaccharides so useful are produced by elaboration of strains of the species *S.mutans* and *S.sanguis*, while the polyfructan which may be incorporated in the vaccine may be an elaboration product of *Streptomyces* Strain SS2.

10 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Brief Summary Text - BSTX:

As indicated hereinabove, animals susceptible to dental caries are immunized, utilizing the present technique, by local administration of a vaccine in the vicinity of the oral mucosa of the animal. The vaccines locally administered by the method hereof may comprise, as the active ingredient thereof, a polysaccharide elaboration product of a cariogenic *Streptococcus* organism, i.e., a polyglucan elaboration product of a strain of *S.mutans* or a strain of *S.sanguis*; or a polyfructan **(levan) elaboration product** of, for example, *Streptococcus* Strain SS2. Alternatively, it has been found that the enzymes

involved in the synthesis of the noted polysaccharides, i.e., **dextransucrase** **and levansucrase**, are effective immunizing agents against caries when administered locally in the vicinity of the oral mucosa in accordance with the present invention.

US-PAT-NO: 3879545

DOCUMENT-IDENTIFIER: US 3879545 A

TITLE: Vaccines for the prevention of dental caries

DATE-ISSUED: April 22, 1975

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|-----------------------------|------------|-------|----------|---------|
| Gaffar; Abdul | Somerset | NJ | 08873 | N/A |
| Kestenbaum; Richard Charles | Old Bridge | NJ | 08857 | N/A |

APPL-NO: 05/ 360964

DATE FILED: May 16, 1973

PARENT-CASE:

REFERENCE TO RELATED APPLICATION This is a continuation-in-part of application Ser. No. 126,933, filed Mar. 22, 1971, now abandoned.

US-CL-CURRENT: 424/244.1; 435/885

ABSTRACT:

Vaccines are prepared from a levan-producing strain of Streptococcus, especially Streptococcus Strain SS2, in the prevention, via immunization, of dental caries. The levan may be used in pure form or may be used in the form of the crude extracellular polysaccharide thereof to produce the vaccine material. Killed Streptococcus SS2 cells containing the extracellular polysaccharide may also be used in lieu of either the pure levan or the polysaccharide material.

9 Claims, 0 Drawing figures

Exemplary Claim Number: 1

----- KWIC -----

Detailed Description Text - DETX:

The enzymes dextransucrase and levansucrase are produced by S. mutans and Streptococcus Strain SS2, and are involved in the synthesis of dextran and levan by such organisms, respectively. These extracellular polysaccharides are believed to have a role in plaque formation and in the consequent development of caries. Accordingly, it was believed that immunization with dextransucrase

or levansucrase might result in the production of antibodies to such enzymes which would neutralize their activity in vivo and thus inhibit synthesis of the polysaccharides and result in decreased plaque formation and lowered incidence of caries. As set forth hereinafter, however, experimentation has failed to confirm this hypothesis.

* * * * * STN Columbus * * * * *

FILE 'HOME' ENTERED AT 15:32:30 ON 08 OCT 2002

=> fil .bec,fsta
COST IN U.S. DOLLARS

180
3.8
218
SINCE FILE TOTAL
ENTRY SESSION
0.21 0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
ESBIOBASE, BIOTECHNO, WPIDS, FSTA' ENTERED AT 15:32:45 ON 08 OCT 2002
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

12 FILES IN THE FILE LIST

=> s fructosyltransferase# or fructosyl transferase# or inulinsucrase# or
levansucrase# or (inulin or levan) (w) sucrase#

FILE 'MEDLINE'

123 FRUCTOSYLTRANSFERASE#
182 FRUCTOSYL
41070 TRANSFERASE#
14 FRUCTOSYL TRANSFERASE#
(FRUCTOSYL (W) TRANSFERASE#)
0 INULINSUCRASE#
221 LEVANSUCRASE#
7368 INULIN
365 LEVAN
2939 SUCRASE#
15 (INULIN OR LEVAN) (W) SUCRASE#
L1 351 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

FILE 'SCISEARCH'

245 FRUCTOSYLTRANSFERASE#
263 FRUCTOSYL
34402 TRANSFERASE#
85 FRUCTOSYL TRANSFERASE#
(FRUCTOSYL (W) TRANSFERASE#)
1 INULINSUCRASE#
273 LEVANSUCRASE#
2647 INULIN
380 LEVAN
1717 SUCRASE#
8 (INULIN OR LEVAN) (W) SUCRASE#
L2 551 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

FILE 'LIFESCI'

98 FRUCTOSYLTRANSFERASE#
78 "FRUCTOSYL"
11631 TRANSFERASE#
21 FRUCTOSYL TRANSFERASE#
("FRUCTOSYL" (W) TRANSFERASE#)
0 INULINSUCRASE#
153 LEVANSUCRASE#
636 INULIN
226 LEVAN
355 SUCRASE#
5 (INULIN OR LEVAN) (W) SUCRASE#
L3 253 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

FILE 'BIOTECHDS'

```

101 FRUCTOSYLTRANSFERASE#
104 FRUCTOSYL
2010 TRANSFERASE#
46 FRUCTOSYL TRANSFERASE#
    (FRUCTOSYL(W) TRANSFERASE#)
0 INULINSUCRASE#
170 LEVANSUCRASE#
381 INULIN
184 LEVAN
73 SUCRASE#
6 (INULIN OR LEVAN) (W) SUCRASE#
L4 295 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
    # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'BIOSIS'

```

266 FRUCTOSYLTRANSFERASE#
410 FRUCTOSYL
66553 TRANSFERASE#
138 FRUCTOSYL TRANSFERASE#
    (FRUCTOSYL(W) TRANSFERASE#)
0 INULINSUCRASE#
258 LEVANSUCRASE#
6385 INULIN
691 LEVAN
3279 SUCRASE#
92 (INULIN OR LEVAN) (W) SUCRASE#
L5 660 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
    # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'EMBASE'

```

114 FRUCTOSYLTRANSFERASE#
104 "FRUCTOSYL"
31217 TRANSFERASE#
9 FRUCTOSYL TRANSFERASE#
    ("FRUCTOSYL" (W) TRANSFERASE#)
1 INULINSUCRASE#
205 LEVANSUCRASE#
6943 INULIN
378 LEVAN
1825 SUCRASE#
6 (INULIN OR LEVAN) (W) SUCRASE#
L6 306 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
    # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'HCAPLUS'

```

442 FRUCTOSYLTRANSFERASE#
581 FRUCTOSYL
41173 TRANSFERASE#
112 FRUCTOSYL TRANSFERASE#
    (FRUCTOSYL(W) TRANSFERASE#)
2 INULINSUCRASE#
470 LEVANSUCRASE#
8416 INULIN
1021 LEVAN
3329 SUCRASE#
61 (INULIN OR LEVAN) (W) SUCRASE#
L7 948 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
    # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'NTIS'

```

2 FRUCTOSYLTRANSFERASE#
2 FRUCTOSYL
975 TRANSFERASE#
0 FRUCTOSYL TRANSFERASE#

```

```

        (FRUCTOSYL(W) TRANSFERASE#)
    0 INULINSUCRASE#
    3 LEVANSUCRASE#
    71 INULIN
    16 LEVAN
    23 SUCRASE#
    0 (INULIN OR LEVAN) (W) SUCRASE#
L8      4 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
        # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'ESBIOBASE'

```

    90 FRUCTOSYLTRANSFERASE#
    105 FRUCTOSYL
    25079 TRANSFERASE#
    32 FRUCTOSYL TRANSFERASE#
        (FRUCTOSYL(W) TRANSFERASE#)
    1 INULINSUCRASE#
    103 LEVANSUCRASE#
    825 INULIN
    114 LEVAN
    418 SUCRASE#
    3 (INULIN OR LEVAN) (W) SUCRASE#
L9      213 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
        # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'BIOTECHNO'

```

    109 FRUCTOSYLTRANSFERASE#
    91 FRUCTOSYL
    14662 TRANSFERASE#
    26 FRUCTOSYL TRANSFERASE#
        (FRUCTOSYL(W) TRANSFERASE#)
    1 INULINSUCRASE#
    187 LEVANSUCRASE#
    798 INULIN
    206 LEVAN
    460 SUCRASE#
    3 (INULIN OR LEVAN) (W) SUCRASE#
L10     291 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
        # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'WPIDS'

```

    25 FRUCTOSYLTRANSFERASE#
    145 FRUCTOSYL
    3683 TRANSFERASE#
    68 FRUCTOSYL TRANSFERASE#
        (FRUCTOSYL(W) TRANSFERASE#)
    0 INULINSUCRASE#
    18 LEVANSUCRASE#
    556 INULIN
    130 LEVAN
    91 SUCRASE#
    26 (INULIN OR LEVAN) (W) SUCRASE#
L11     119 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
        # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

```

FILE 'FSTA'

```

    51 FRUCTOSYLTRANSFERASE#
    102 FRUCTOSYL
    1786 TRANSFERASE#
    30 FRUCTOSYL TRANSFERASE#
        (FRUCTOSYL(W) TRANSFERASE#)
    0 INULINSUCRASE#
    83 LEVANSUCRASE#
    621 INULIN

```

129 LEVAN
 82 SUCRASE#
 4 (INULIN OR LEVAN) (W) SUCRASE#
 L12 156 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
 # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

TOTAL FOR ALL FILES

L13 4147 FRUCTOSYLTRANSFERASE# OR FRUCTOSYL TRANSFERASE# OR INULINSUCRASE
 # OR LEVANSUCRASE# OR (INULIN OR LEVAN) (W) SUCRASE#

=> s l13 and (lactobacillus or lactic acid bacteri?)

FILE 'MEDLINE'

9990 LACTOBACILLUS
 26300 LACTIC
 1139532 ACID
 510682 BACTERI?
 1533 LACTIC ACID BACTERI?
 (LACTIC(W)ACID(W)BACTERI?)
 L14 3 L1 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'SCISEARCH'

8761 LACTOBACILLUS
 18710 LACTIC
 873438 ACID
 268820 BACTERI?
 4981 LACTIC ACID BACTERI?
 (LACTIC(W)ACID(W)BACTERI?)
 L15 5 L2 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'LIFESCI'

4975 LACTOBACILLUS
 5856 "LACTIC"
 250534 "ACID"
 153031 BACTERI?
 2196 LACTIC ACID BACTERI?
 ("LACTIC" (W) "ACID" (W) BACTERI?)
 L16 4 L3 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOTECHDS'

2245 LACTOBACILLUS
 4736 LACTIC
 89817 ACID
 95123 BACTERI?
 2446 LACTIC ACID BACTERI?
 (LACTIC(W)ACID(W)BACTERI?)
 L17 7 L4 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOSIS'

14452 LACTOBACILLUS
 25647 LACTIC
 1084348 ACID
 741268 BACTERI?
 4472 LACTIC ACID BACTERI?
 (LACTIC(W)ACID(W)BACTERI?)
 L18 5 L5 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'EMBASE'

7409 LACTOBACILLUS
 30639 "LACTIC"
 1097905 "ACID"
 370122 BACTERI?
 1658 LACTIC ACID BACTERI?
 ("LACTIC" (W) "ACID" (W) BACTERI?)
 L19 8 L6 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

```

FILE 'HCAPLUS'
    17489 LACTOBACILLUS
    76819 LACTIC
    3447037 ACID
    474557 BACTERI?
    7403 LACTIC ACID BACTERI?
        (LACTIC(W)ACID(W)BACTERI?)
L20      10 L7 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'NTIS'
    109 LACTOBACILLUS
    559 LACTIC
    42751 ACID
    17900 BACTERI?
    33 LACTIC ACID BACTERI?
        (LACTIC(W)ACID(W)BACTERI?)
L21      0 L8 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'ESBIOBASE'
    2897 LACTOBACILLUS
    4120 LACTIC
    235097 ACID
    128962 BACTERI?
    1526 LACTIC ACID BACTERI?
        (LACTIC(W)ACID(W)BACTERI?)
L22      3 L9 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'BIOTECHNO'
    4417 LACTOBACILLUS
    7301 LACTIC
    311833 ACID
    168151 BACTERI?
    1790 LACTIC ACID BACTERI?
        (LACTIC(W)ACID(W)BACTERI?)
L23      6 L10 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'WPIDS'
    2846 LACTOBACILLUS
    12165 LACTIC
    760077 ACID
    81064 BACTERI?
    1833 LACTIC ACID BACTERI?
        (LACTIC(W)ACID(W)BACTERI?)
L24      6 L11 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

FILE 'FSTA'
    8491 LACTOBACILLUS
    13939 LACTIC
    105845 ACID
    57781 BACTERI?
    5986 LACTIC ACID BACTERI?
        (LACTIC(W)ACID(W)BACTERI?)
L25      2 L12 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

TOTAL FOR ALL FILES
L26      59 L13 AND (LACTOBACILLUS OR LACTIC ACID BACTERI?)

=> s (fructan or levan or inulin) (5a) (mak##### or produc? or synthes?)
FILE 'MEDLINE'
    146 FRUCTAN
    365 LEVAN
    7368 INULIN
    223823 MAK#####

```


1033764 PRODUC?
410025 SYNTHES?
L27 214 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'SCISEARCH'

543 FRUCTAN
380 LEVAN
2647 INULIN
246085 MAK#####
1373146 PRODUC?
704015 SYNTHES?
L28 404 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'LIFESCI'

113 FRUCTAN
226 LEVAN
636 INULIN
42225 MAK#####
427634 PRODUC?
122575 SYNTHES?
L29 209 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'BIOTECHDS'

77 FRUCTAN
184 LEVAN
381 INULIN
7187 MAK#####
166903 PRODUC?
23309 SYNTHES?
L30 267 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'BIOSIS'

725 FRUCTAN
691 LEVAN
6385 INULIN
153727 MAK#####
1426196 PRODUC?
567645 SYNTHES?
L31 620 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'EMBASE'

305 FRUCTAN
378 LEVAN
6943 INULIN
198540 MAK#####
1008789 PRODUC?
503381 SYNTHES?
L32 238 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'HCAPLUS'

988 FRUCTAN
1021 LEVAN
8416 INULIN
459429 MAK#####
3535563 PRODUC?
722531 PRODN
3891607 PRODUC?
(PRODUC? OR PRODN)

1247505 SYNTHES?
L33 1121 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'NTIS'

3 FRUCTAN
16 LEVAN
71 INULIN
113045 MAK#####
352744 PRODUC?
40713 SYNTHES?
L34 5 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'ESBIOBASE'

226 FRUCTAN
114 LEVAN
825 INULIN
45168 MAK#####
388719 PRODUC?
137015 SYNTHES?
L35 180 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'BIOTECHNO'

197 FRUCTAN
206 LEVAN
798 INULIN
29901 MAK#####
355179 PRODUC?
155509 SYNTHES?
L36 192 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'WPIDS'

118 FRUCTAN
130 LEVAN
556 INULIN
531367 MAK#####
1852429 PRODUC?
100121 SYNTHES?
L37 151 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

FILE 'FSTA'

128 FRUCTAN
129 LEVAN
621 INULIN
16051 MAK#####
258556 PRODUC?
10231 SYNTHES?
L38 267 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

TOTAL FOR ALL FILES

L39 3868 (FRUCTAN OR LEVAN OR INULIN) (5A) (MAK##### OR PRODUC? OR SYNTHES
?)

=> s 113 and 139

FILE 'MEDLINE'

L40 68 L1 AND L27

FILE 'SCISEARCH'

L41 143 L2 AND L28

FILE 'LIFESCI'
 L42 60 L3 AND L29

 FILE 'BIOTECHDS'
 L43 64 L4 AND L30

 FILE 'BIOSIS'
 L44 178 L5 AND L31

 FILE 'EMBASE'
 L45 59 L6 AND L32

 FILE 'HCAPLUS'
 L46 243 L7 AND L33

 FILE 'NTIS'
 L47 0 L8 AND L34

 FILE 'ESBIOBASE'
 L48 79 L9 AND L35

 FILE 'BIOTECHNO'
 L49 78 L10 AND L36

 FILE 'WPIDS'
 L50 23 L11 AND L37

 FILE 'FSTA'
 L51 48 L12 AND L38

 TOTAL FOR ALL FILES
 L52 1043 L13 AND L39

 => s 113 (5a) l39
 FILE 'MEDLINE'
 L53 27 L1 (5A) L27

 FILE 'SCISEARCH'
 L54 44 L2 (5A) L28

 FILE 'LIFESCI'
 L55 18 L3 (5A) L29

 FILE 'BIOTECHDS'
 L56 24 L4 (5A) L30

 FILE 'BIOSIS'
 L57 65 L5 (5A) L31

 FILE 'EMBASE'
 L58 21 L6 (5A) L32

 FILE 'HCAPLUS'
 L59 93 L7 (5A) L33

 FILE 'NTIS'
 L60 0 L8 (5A) L34

 FILE 'ESBIOBASE'
 L61 27 L9 (5A) L35

 FILE 'BIOTECHNO'
 L62 27 L10 (5A) L36

```
FILE 'WPIDS'
L63          13 L11(5A)L37

FILE 'FSTA'
L64          22 L12(5A)L38

TOTAL FOR ALL FILES
L65          381 L13(5A) L39

=> s (l26 or l65) not 2002/py
FILE 'MEDLINE'
          363372 2002/PY
L66          26 (L14 OR L53) NOT 2002/PY

FILE 'SCISEARCH'
          637035 2002/PY
L67          43 (L15 OR L54) NOT 2002/PY

FILE 'LIFESCI'
          32374 2002/PY
L68          22 (L16 OR L55) NOT 2002/PY

FILE 'BIOTECHDS'
          6836 2002/PY
L69          31 (L17 OR L56) NOT 2002/PY

FILE 'BIOSIS'
          283018 2002/PY
L70          69 (L18 OR L57) NOT 2002/PY

FILE 'EMBASE'
          272986 2002/PY
L71          24 (L19 OR L58) NOT 2002/PY

FILE 'HCAPLUS'
          739015 2002/PY
L72          92 (L20 OR L59) NOT 2002/PY

FILE 'NTIS'
          4714 2002/PY
L73          0 (L21 OR L60) NOT 2002/PY

FILE 'ESBIOBASE'
          189072 2002/PY
L74          26 (L22 OR L61) NOT 2002/PY

FILE 'BIOTECHNO'
          77469 2002/PY
L75          28 (L23 OR L62) NOT 2002/PY

FILE 'WPIDS'
          662514 2002/PY
L76          15 (L24 OR L63) NOT 2002/PY

FILE 'FSTA'
          12564 2002/PY
L77          21 (L25 OR L64) NOT 2002/PY

TOTAL FOR ALL FILES
L78          397 (L26 OR L65) NOT 2002/PY

=> dup rem l78
PROCESSING COMPLETED FOR L78
```

L79 187 DUP REM L78 (210 DUPLICATES REMOVED)

=> save temp inulase/a l79
ANSWER SET L79 HAS BEEN SAVED AS 'INULASE/A'

=> d tot

L66 ANSWER 1 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI New enzymes having **fructosyltransferase** activity (e.g.
inulosucrase or **levansucrase**), useful for producing useful
levans, inulins and fructo-oligosaccharides from sucrose, which are
particularly useful as prebiotic substrates;
recombinant protein production via plasmid expression in host cell
useful for fructo-oligosaccharide inulin-type or levan-type production
AU VAN GEEL-SCHUTTEN G H; RAHAOUI H; DIJKHUIZEN L; VAN HIJUM S A F T
AN 2002-05167 BIOTECHDS
PI WO 2001090319 29 Nov 2001

L66 ANSWER 2 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Producing difructose dianhydride IV from sucrose, involves reacting sugar
solution in the presence of **levansucrase** to **produce**
levan, and reacting **levan** solution in the presence of
levan-fructotransferase to produce DFA IV;
involving vector plasmid pUDFA81-mediated gene transfer for expression
in Escherichia coli
AU Rhee S; Song K; Kim C; Ryu E; Lee Y
AN 2001-10802 BIOTECHDS
PI WO 2001029185 26 Apr 2001

L66 ANSWER 3 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Purification, characterization and use of inulosucrase and
levansucrase from **Lactobacillus reuteri**
SO PCT Int. Appl., 54 pp.
CODEN: PIXXD2
IN Van Geel-Schutten, Gerritdina Hendrika; Rahaoui, Hakim; Dijkhuizen,
Lubbert; Van Hijum, Sacha Adrianus Fokke Taco
AN 2001:868644 HCAPLUS
DN 136:17259

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------|--|----------|-----------------|----------|
| ----- | ---- | ----- | ----- | ----- |
| PI WO 2001090319 | A2 | 20011129 | WO 2001-NL392 | 20010523 |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |

L66 ANSWER 4 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Enzymatic production of difructose dianhydride IV from sucrose and
relevant enzymes and genes coding for them
SO PCT Int. Appl., 72 pp.
CODEN: PIXXD2
IN Rhee, Sangki; Song, Kibang; Kim, Chulho; Ryu, Eunja; Lee, Yongbok
AN 2001:300842 HCAPLUS
DN 134:339638

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------|---|----------|-----------------|----------|
| ----- | ---- | ----- | ----- | ----- |
| PI WO 2001029185 | A1 | 20010426 | WO 2000-KR1183 | 20001019 |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, | | | |

HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU,
 LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD,
 SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU,
 ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
 DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
 CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
 EP 1141236 A1 20011010 EP 2000-976414 20001019
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO

- L66 ANSWER 5 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Roles of fructosyltransferase and levanase-sucrase of *Actinomyces*
naeslundii in fructan and sucrose metabolism
 SO Infection and Immunity (2001), 69(9), 5395-5402
 CODEN: INFIBR; ISSN: 0019-9567
 AU Bergeron, Lori J.; Burne, Robert A.
 AN 2001:642791 HCAPLUS
 DN 135:284996
- L66 ANSWER 6 OF 187 MEDLINE DUPLICATE 1
 TI Sucrose metabolism in plastids.
 SO PLANT PHYSIOLOGY, (2001 Feb) 125 (2) 926-34.
 Journal code: 0401224. ISSN: 0032-0889.
 AU Gerrits N; Turk S C; van Dun K P; Hulleman S H; Visser R G; Weisbeek P J;
 Smeekens S C
 AN 2002006770 IN-PROCESS
- L66 ANSWER 7 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 2
 TI Polymerase and hydrolase activities of *Zymomonas mobilis* levansucrase
 separately modulated by in vitro mutagenesis and elevated temperature
 SO PROCESS BIOCHEMISTRY, (JAN 2001) Vol. 36, No. 6, pp. 543-548.
 Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON,
 OXFORD OX5 1GB, OXON, ENGLAND.
 ISSN: 0032-9592.
 AU Sangiliyandi G (Reprint); Gunasekaran P
 AN 2001:110312 SCISEARCH
- L66 ANSWER 8 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 3
 TI Levan-producing *Bacillus subtilis* BS 62 and its phylogeny based on its 16S
 rDNA sequence
 SO JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY, (JUN 2001) Vol. 11, No. 3, pp.
 428-434.
 Publisher: KOREAN SOC APPLIED MICROBIOLOGY, KOREA SCI TECHNOL CENTER #507,
 635-4 YEOGSAM-DONG, KANGNAM-GU, SEOUL 135-703, SOUTH KOREA.
 ISSN: 1017-7825.
 AU Choi S H; Sung C; Choi W Y (Reprint)
 AN 2001:547396 SCISEARCH
- L66 ANSWER 9 OF 187 MEDLINE DUPLICATE 4
 TI Gene cloning and functional characterization by heterologous expression of
 the fructosyltransferase of *Aspergillus sydowi* IAM 2544.
 SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2001 Jan) 67 (1) 363-70.
 Journal code: 7605801. ISSN: 0099-2240.
 AU Heyer A G; Wendenburg R
 AN 2001256590 MEDLINE
- L66 ANSWER 10 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 5
 TI **Levan production** by use of the recombinant
levansucrase immobilized on titanium-activated magnetite
 SO PROCESS BIOCHEMISTRY, (10 DEC 2001) Vol. 37, No. 4, pp. 339-343.
 Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON,
 OXFORD OX5 1GB, OXON, ENGLAND.
 ISSN: 0032-9592.

AU Jang K H; Song K B; Park B S; Kim C H; Chung B H; Choue R W; Lee K S; Lee C; Chun U H; Rhee S K (Reprint)
AN 2001:997655 SCISEARCH

L66 ANSWER 11 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 6
TI Comparison of characteristics of **levan produced** by
different preparations of **levansucrase** from *Zymomonas mobilis*
SO BIOTECHNOLOGY LETTERS, (MAR 2001) Vol. 23, No. 5, pp. 339-344.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0141-5492.

AU Jang K H; Song K B; Kim C H; Chung B H; Kang S A; Chun U H; Choue R W; Rhee S K (Reprint)
AN 2001:159661 SCISEARCH

L66 ANSWER 12 OF 187 MEDLINE DUPLICATE 7
TI Purification of a novel **fructosyltransferase** from
Lactobacillus reuteri strain 121 and characterization of the levan produced.
SO FEMS MICROBIOLOGY LETTERS, (2001 Dec 18) 205 (2) 323-8.
Journal code: 7705721. ISSN: 0378-1097.

AU van Hijum S A; Bonting K; van der Maarel M J; Dijkhuizen L
AN 2002003002 MEDLINE

L66 ANSWER 13 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Purification of a novel **fructosyltransferase** from
Lactobacillus reuteri strain 121 and characterization of the levan produced;
with application in food and feedstuff
SO FEMS Microbiol.Lett.; (2001) 205, 2, 323-28
CODEN: FMLED7 ISSN: 0378-1097

AU van Hijum S A F T; Bonting K; van der Maarel M J E C; *Dijkhuizen L
AN 2001-15551 BIOTECHDS

L66 ANSWER 14 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI Regulation of fructosyltransferase expression in *Streptococcus mutans* by a two-component regulatory system.
SO Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 78. <http://www.asmta.org/mtgsrc/generalmeeting.htm>. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.

AU Delaney, G. D. (1); Lee, S. F. (1)
AN 2002:176442 BIOSIS

L66 ANSWER 15 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)
TI Molecular structure of the **Lactobacillus** plantarum sucrose utilization locus: Comparison with *Pediococcus pentosaceus*
SO MOLECULAR BIOLOGY, (JAN-FEB 2001) Vol. 35, No. 1, pp. 15-22.
Publisher: CONSULTANTS BUREAU, 233 SPRING ST, NEW YORK, NY 10013 USA.
ISSN: 0026-8933.

AU Naumoff D G (Reprint); Livshits V A
AN 2001:245117 SCISEARCH

L66 ANSWER 16 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Homopolysaccharides from **lactic acid bacteria**
SO International Dairy Journal, (2001), 11 (9) 675-685, many ref.
ISSN: 0958-6946

AU Monsan, P.; Bozonnet, S.; Alberne, C.; Joucla, G.; Willemot, R. M.; Remaud-Simeon, M.
AN 2002:A0039 FSTA

L66 ANSWER 17 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI Comparison of characteristics of **levan produced** by
 different preparations of **levansucrase** from *Zymomonas mobilis*.
 SO Biotechnology Letters, (2001), 23 (5) 339-344, 18 ref.
 ISSN: 0141-5492
 AU Ki-Hyo Jang; Ki-Bang Song; Chul Ho Kim; Bong Hyun Chung; Soon Ah Kang;
 Uck-Han Chun; Ryo Won Choue; Sang-Ki Rhee
 AN 2001(07):B1020 FSTA

L66 ANSWER 18 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI Gene cloning and functional characterization by heterologous expression of
 the fructosyltransferase of *Aspergillus sydowi* IAM 2544.
 SO Applied and Environmental Microbiology, (2001), 67 (1) 363-370, 48 ref.
 ISSN: 0099-2240
 AU Heyer, A. G.; Wendenburg, R.
 AN 2001(04):B0563 FSTA

L66 ANSWER 19 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI **Levan production** by use of the recombinant
levansucrase immobilized on titanium-activated magnetite.
 SO Process Biochemistry, (2001), 37 (4) 339-343, 13 ref.
 ISSN: 0032-9592
 AU Ki-Hyo Jang; Ki-Bang Song; Beum-Seek Park; Chul Ho Kim; Bong Hyun Chung;
 Ryo Won Choue; Kwang Suck Lee; Chan Lee; Uck-Han Chun; Sang-Ki Rhee
 AN 2002:B0928 FSTA

L66 ANSWER 20 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI DNA sequences encoding carbohydrate polymer synthesizing enzymes and
 method for producing transgenic plants.
 SO Official Gazette of the United States Patent and Trademark Office Patents,
 (May 2, 2000) Vol. 1234, No. 1, pp. No pagination. e-file.
 ISSN: 0098-1133.
 AU Kooops, Andries Jurriaan (1); van der Meer, Ingrid Maria; Van Tunen, Arjen
 Johannes
 AN 2000:490325 BIOSIS

L66 ANSWER 21 OF 187 MEDLINE DUPLICATE 8
 TI Transgenic potato (*Solanum tuberosum*) tubers synthesize the full spectrum
 of inulin molecules naturally occurring in globe artichoke (*Cynara
 scolymus*) roots.
 SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
 AMERICA, (2000 Jul 18) 97 (15) 8699-704.
 Journal code: 7505876. ISSN: 0027-8424.
 AU Hellwege E M; Czapla S; Jahnke A; Willmitzer L; Heyer A G
 AN 2000406971 MEDLINE

L66 ANSWER 22 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Globular shape of high molar mass inulin revealed by static light
 scattering and viscometry
 SO Polymer (2000), 41(22), 8009-8016
 CODEN: POLMAG; ISSN: 0032-3861
 AU Wolff, D.; Czapla, S.; Heyer, A. G.; Radosta, S.; Mischnick, P.; Springer,
 J.
 AN 2000:449650 HCAPLUS
 DN 133:224500

L66 ANSWER 23 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Characterization of the fructosyltransferase gene of *Actinomyces
 naeslundii* WVU45
 SO Journal of Bacteriology (2000), 182(13), 3649-3654
 CODEN: JOBAAY; ISSN: 0021-9193
 AU Bergeron, Lori J.; Morou-Bermudez, Evangelia; Burne, Robert A.
 AN 2000:446271 HCAPLUS
 DN 133:318095

L66 ANSWER 24 OF 187 MEDLINE DUPLICATE 9
 TI Drought induces **fructan synthesis** and 1-SST
 (sucrose:sucrose **fructosyltransferase**) in roots and leaves of
 chicory seedlings (*Cichorium intybus* L.).
 SO PLANTA, (2000 Apr) 210 (5) 808-14.
 Journal code: 1250576. ISSN: 0032-0935.
 AU De Roover J; Vandenbranden; Van Laere A; Van den Ende W
 AN 2000263203 MEDLINE

L66 ANSWER 25 OF 187 MEDLINE DUPLICATE 10
 TI Fructan accumulation induced by nitrogen deficiency in barley leaves
 correlates with the level of sucrose:fructan 6-fructosyltransferase mRNA.
 SO PLANTA, (2000 Oct) 211 (5) 701-7.
 Journal code: 1250576. ISSN: 0032-0935.
 AU Wang C; Van den Ende W; Tillberg J E
 AN 2001135835 MEDLINE

L66 ANSWER 26 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI Polymerase and hydrolase activities of *Zymomonas mobilis* levansucrase
 separately modulated by in vitro mutagenesis and elevated temperature;
 involving vector plasmid pLSS41-mediated levansucrase gene transfer
 for expression in *Escherichia coli*
 SO Process Biochem.; (2000) 36, 6, 543-48
 CODEN: PBCHE5 ISSN: 0032-9592
 AU Sangiliyandi G; Gunasekaran P
 AN 2001-03020 BIOTECHDS

L66 ANSWER 27 OF 187 MEDLINE DUPLICATE 11
 TI Disaccharide-mediated regulation of sucrose:fructan-6-
fructosyltransferase, a key enzyme of **fructan**
synthesis in barley leaves.
 SO PLANT PHYSIOLOGY, (2000 May) 123 (1) 265-74.
 Journal code: 0401224. ISSN: 0032-0889.
 AU Muller J; Aeschbacher R A; Sprenger N; Boller T; Wiemken A
 AN 2000407029 MEDLINE

L66 ANSWER 28 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Cloning and sequencing of the levansucrase gene from *Acetobacter xylinum*
 NCI 1005
 SO DNA Research (2000), 7(4), 237-242
 CODEN: DARSE8; ISSN: 1340-2838
 AU Tajima, Kenji; Tanio, Toshiyuki; Kobayashi, Yasunobu; Kohno, Hiroyuki;
 Fujiwara, Masashi; Shiba, Toshikazu; Erata, Tomoki; Munekata, Masanobu;
 Takai, Mitsuo
 AN 2000:647932 HCAPLUS
 DN 134:52200

L66 ANSWER 29 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 DUPLICATE 12
 TI Exopolysaccharide production by *Lactobacillus reuteri*, involving
 sucrase type of enzymes.
 SO Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische
 Wetenschappen Universiteit Gent, (2000) Vol. 65, No. 3A, pp. 197-201.
 print.
 AU van Geel-Schutten, G. H. (1); van Hijum, S. A. F. T.; Kralj, S.; Rahaoui,
 H. (1); Leer, R. J. (1); Dijkhuizen, L.
 AN 2001:93723 BIOSIS

L66 ANSWER 30 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Strategies for fructan production in transgenic sugarcane (*Saccharum* spp
 L.) and sweet potato (*Ipomoea batata* L.) plants expressing the *Acetobacter*
diazotrophicus levansucrase
 SO Developments in Plant Genetics and Breeding (2000), 5(Plant Genetic

Engineering Towards the Third Millennium), 194-198

CODEN: DPGBD6; ISSN: 0168-7972

AU Trujillo, L. E.; Arrieta, J. G.; Enriquez, G. A.; Dafhnis, F.; Fuentes, A.
D.; Garcia, R.; Soto, M.; Alfonso, D.; Banguela, A.; Hernandez, L.
AN 2000:338870 HCAPLUS
DN 134:96023

L66 ANSWER 31 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Exopolysaccharides of *Erwinia amylovora*: structure, biosynthesis,
regulation, role in pathogenicity of amylovoran and levan
SO Fire Blight (2000), 117-140. Editor(s): Vanneste, Joel. Publisher: CABI
Publishing, Wallingford, UK.
CODEN: 69CPES; ISBN: 0-85199-294-3
AU Geider, Klaus
AN 2002:375980 HCAPLUS
DN 137:152065

L66 ANSWER 32 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 13
TI **Production of levan** using recombinant
levansucrase immobilized on hydroxyapatite
SO BIOPROCESS ENGINEERING, (JUL 2000) Vol. 23, No. 1, pp. 89-93.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0178-515X.
AU Jang K H; Song K B; Kim J S; Kim C H; Chung B H; Rhee S K (Reprint)
AN 2000:607559 SCISEARCH

L66 ANSWER 33 OF 187 HCAPLUS COPYRIGHT 2002 ACS DUPLICATE 14
TI A novel fructosyl transferase isolated from artichoke, and its use in the
production of long-chain inulin
SO PCT Int. Appl., 50 pp.
CODEN: PIXXD2
IN Heyer, Arnd G.; Hellwege, Elke W.; Gritscher, Dominique
AN 1999:326065 HCAPLUS
DN 130:348208

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|--|----------|------------------|----------|
| ----- | --- | ----- | ----- | ----- |
| PI WO 9924593 | A1 | 19990520 | WO 1998-EP7115 | 19981106 |
| W: | AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| DE 19749122 | A1 | 19990610 | DE 1997-19749122 | 19971106 |
| ZA 9810110 | A | 19990505 | ZA 1998-10110 | 19981105 |
| CA 2309133 | AA | 19990520 | CA 1998-2309133 | 19981106 |
| AU 9916674 | A1 | 19990531 | AU 1999-16674 | 19981106 |
| EP 1029067 | A1 | 20000823 | EP 1998-961152 | 19981106 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | |
| BR 9813968 | A | 20000926 | BR 1998-13968 | 19981106 |
| JP 2001521757 | T2 | 20011113 | JP 2000-519587 | 19981106 |

L66 ANSWER 34 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Transgenic plants comprising 1-SST and 1-FFT **fructosyltransferase**
genes for a modified **inulin production** profile
SO Eur. Pat. Appl., 55 pp.
CODEN: EPXXDW
IN Koops, Andries Jurriaan; Sevenier, Robert; Van Tunen Arjen, Johannes; De
Leenheer, Lena
AN 1999:690873 HCAPLUS
DN 131:318572

| | PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|-----|--|------|----------|-----------------|----------|
| PI | EP 952222 | A1 | 19991027 | EP 1998-870084 | 19980417 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| WO | 9954480 | A1 | 19991028 | WO 1999-EP2538 | 19990415 |
| | W: AU, BR, BY, CA, CN, CZ, EE, GE, HU, ID, IN, JP, LT, LV, NZ, PL, RO, RU, SI, SK, UA, US, ZA | | | | |
| | RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| AU | 9936065 | A1 | 19991108 | AU 1999-36065 | 19990415 |
| EP | 1084254 | A1 | 20010321 | EP 1999-917984 | 19990415 |
| | R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, PT, IE, SI, LT, LV, RO | | | | |
| L66 | ANSWER 35 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 15 | | | | |
| TI | Biochemical and structural characterization of the glucan and fructan exopolysaccharides synthesized by the Lactobacillus reuteri wild-type strain and by mutant strains | | | | |
| SO | APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (JUL 1999) Vol. 65, No. 7, pp. 3008-3014. Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171. ISSN: 0099-2240. | | | | |
| AU | VanGeelschutten G H; Faber E J; Smit E; Bonting K; Smith M R; TenBrink B; Kamerling J P; Vliegthart J F G; Dijkhuizen L (Reprint) | | | | |
| AN | 1999:530279 SCISEARCH | | | | |
| L66 | ANSWER 36 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 16 | | | | |
| TI | Purification and immobilization of fructosyl transferase for production of fructo-oligosaccharide(s) from sucrose. | | | | |
| SO | Indian Journal of Experimental Biology, (1999) 37/8 (830-834). Refs: 16 ISSN: 0019-5189 CODEN: IJEBA6 | | | | |
| AU | Patil V.B.; Patil N.B. | | | | |
| AN | 1999274169 EMBASE | | | | |
| L66 | ANSWER 37 OF 187 HCAPLUS COPYRIGHT 2002 ACS | | | | |
| TI | Porous membranes with functional graft copolymer surfaces for enzyme membrane reactors | | | | |
| SO | Werkstoffwoche '98, Band VIII: Symposium 10, Polymere; Symposium 14, Simulation Polymere, Munich, Sept., 1998 (1999), Meeting Date 1998, 223-228. Editor(s): Michaeli, Walter. Publisher: Wiley-VCH Verlag GmbH, Weinheim, Germany. CODEN: 68SRAZ | | | | |
| AU | Ulbricht, Mathias; Hicke, Hans-Georg | | | | |
| AN | 2000:193601 HCAPLUS | | | | |
| DN | 133:136256 | | | | |
| L66 | ANSWER 38 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 17 | | | | |
| TI | In vitro digestibility and fermentability of levan and its hypocholesterolemic effects in rats | | | | |
| SO | JOURNAL OF NUTRITIONAL BIOCHEMISTRY, (JAN 1999) Vol. 10, No. 1, pp. 13-18. Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010. ISSN: 0955-2863. | | | | |
| AU | Yamamoto Y; Takahashi Y; Kawano M; Iizuka M; Matsumoto T; Saeki S; Yamaguchi H (Reprint) | | | | |
| AN | 1999:81783 SCISEARCH | | | | |
| L66 | ANSWER 39 OF 187 FSTA COPYRIGHT 2002 IFIS | | | | |
| TI | Biochemical and structural characterization of the glucan and fructan exopolysaccharides synthesized by the Lactobacillus reuteri wild-type strain and by mutant strains. | | | | |

SO Applied and Environmental Microbiology, (1999), 65 (7) 3008-3014, 50 ref.
ISSN: 0099-2240

AU Geel-Schutten, G. H. van; Faber, E. J.; Smit, E.; Bonting, K.; Smith, M.
R.; Brink, B. ten; Kamerling, J. P.; Vliegenthart, J. F. G.; Dijkhuizen,
L.

AN 1999(10):A1725 FSTA

L66 ANSWER 40 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Fructan: more than a reserve carbohydrate?
SO Plant Physiology, (1999), 120 (2) 351-359, 43 ref.
ISSN: 0032-0889
AU Vijn, I.; Smeekens, S.
AN 2000(01):L0033 FSTA

L66 ANSWER 41 OF 187 WPIDS (C) 2002 THOMSON DERWENT
TI Production of kojic acid fructoside compounds used as medicines, etc. -
comprises reacting levan sucrase with mixture of kojic acid and fructose.
PI JP 10099091 A 19980421 (199826)* 6p C12P019-44

L66 ANSWER 42 OF 187 MEDLINE DUPLICATE 18
TI Cloning, nucleotide sequence, and expression in Escherichia coli of
levansucrase genes from the plant pathogens Pseudomonas syringae pv.
glycinea and P. syringae pv. phaseolicola.
SO APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1998 Sep) 64 (9) 3180-7.
Journal code: 7605801. ISSN: 0099-2240.
AU Hettwer U; Jaeckel F R; Boch J; Meyer M; Rudolph K; Ullrich M S
AN 1998394981 MEDLINE

L66 ANSWER 43 OF 187 MEDLINE DUPLICATE 19
TI Cloning of sucrose:sucrose 1-**fructosyltransferase** from onion and
synthesis of structurally defined **fructan** molecules from
sucrose.
SO PLANT PHYSIOLOGY, (1998 Aug) 117 (4) 1507-13.
Journal code: 0401224. ISSN: 0032-0889.
AU Vijn I; van Dijken A; Luscher M; Bos A; Smeets E; Weisbeek P; Wiemken A;
Smeekens S
AN 1998369129 MEDLINE

L66 ANSWER 44 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 20
TI Description of the bacterium causing blight of leek as Pseudomonas
syringae pv. porri (pv. nov.)
SO PHYTOPATHOLOGY, (AUG 1998) Vol. 88, No. 8, pp. 844-850.
Publisher: AMER PHYTOPATHOLOGICAL SOC, 3340 PILOT KNOB ROAD, ST PAUL, MN
55121.
ISSN: 0031-949X.
AU Samson R (Reprint); Shafik H; Benjama A; Gardan L
AN 1998:580970 SCISEARCH

L66 ANSWER 45 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI High level fructan accumulation in a transgenic sugar beet;
sugarbeet transgenic plant construction using Jerusalem artichoke
sucrose:sucrose-1-beta-D-**fructosyl-transferase**
expression in taproot to **produce fructan**, useful
as sweetener
SO Nat.Biotechnol.; (1998) 16, 9, 843-46
CODEN: NABIF ISSN: 1087-0156
AU Sevenier R; Hall R D; van der Meer I M; Hakkert H J C; van Tunen A J;
*Koops A J
AN 1999-12234 BIOTECHDS

L66 ANSWER 46 OF 187 MEDLINE DUPLICATE 21
TI Increased cellulose production from sucrose with reduced levan
accumulation by an Acetobacter strain harboring a recombinant plasmid.
SO BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY, (1998 May) 62 (5) 833-6.

Journal code: 9205717. ISSN: 0916-8451.

AU Tonouchi N; Yanase H; Kojima Y; Tsuchida T; Yoshinaga F; Horinouchi S
AN 1998312017 MEDLINE

L66 ANSWER 47 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Covalent enzyme immobilization in different porous polymer membranes
SO Progress in Biotechnology (1998), 15(Stability and Stabilization of
Biocatalysis), 661-666
CODEN: PBITE3; ISSN: 0921-0423
AU Hicke, H.-G.; Becker, M.; Malsch, G.; Ulbricht, M.
AN 1999:159997 HCAPLUS
DN 130:334661

L66 ANSWER 48 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI **Synthesis** of high molecular **inulin** by using purified
fructosyltransferase.
SO Starch, (Sept., 1998) Vol. 50, No. 9, pp. 366-367.
Meeting Info.: 49th Starch Convention Detmold, Germany 1998
ISSN: 0038-9056.
AU Radosta, S. (1); Haberer, M. (1); Heyer, A.; Vorwerk, W.; Wolff, D.
AN 1999:140490 BIOSIS

L66 ANSWER 49 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Structure of the enzymically synthesized fructan inulin
SO Carbohydrate Research (1998), 313(3-4), 165-174
CODEN: CRBRAT; ISSN: 0008-6215
AU Heyer, A. G.; Schroeer, B.; Radosta, S.; Wolff, D.; Czapla, S.; Springer, J.
AN 1999:227288 HCAPLUS
DN 131:29183

L66 ANSWER 50 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Cloning of sucrose:sucrose 1-**fructosyltransferase** from onion and
synthesis of structurally defined **fructan** molecules from
sucrose.
SO Plant Physiology, (1998), 117 (4) 1507-1513, 23 ref.
ISSN: 0032-0889
AU Vijn, I.; Dijken, A. van; Luescher, M.; Bos, A.; Smeets, E.; Weisbeek, P.;
Wiemken, A.; Smeekens, S.
AN 1998(12):J2814 FSTA

L66 ANSWER 51 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Fermentation strategy to enhance plasmid stability during the cultivation
of Escherichia coli for the production of recombinant levansucrase.
SO Journal of Fermentation and Bioengineering, (1998), 86 (4) 391-394, 11
ref.
AU Chul Ho Kim; Jang Young Lee; Min Gon Kim; Ki Bang Song; Jeong Woo Seo;
Bong Hyun Chung; Soon Jae Chang; Sang Ki Rhee
AN 1999(06):B0710 FSTA

L66 ANSWER 52 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Fructan metabolism in chicory roots (Cichorium intybus L.).
SO Dissertation Abstracts International, C, (1997, thesis publ. 1996), 58 (1)
117-118 173pp.
ISSN: 0307-6075
AU Ende, W. van den
AN 1997(09):H0149 FSTA

L66 ANSWER 53 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Novel cellulose-producing bacteria
SO PCT Int. Appl., 51 pp.
CODEN: PIXXD2
IN Tsuchida, Takayasu; Tonouchi, Naoto; Seto, Akira; Kojima, Yukiko;
Matsuoka, Masanobu; Yoshinaga, Fumihiko

AN 1997:717991 HCAPLUS

DN 127:328870

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| WO 9740135 | A1 | 19971030 | WO 1997-JP514 | 19970224 |
| W: JP, KR, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 834555 | A1 | 19980408 | EP 1997-904620 | 19970224 |
| R: DE, FR, GB | | | | |
| US 5962278 | A | 19991005 | US 1998-973757 | 19980203 |
| US 6110712 | A | 20000829 | US 1999-274470 | 19990323 |

L66 ANSWER 54 OF 187 WPIDS (C) 2002 THOMSON DERWENT

TI Acetic acid bacterium transformed to improve sucrose-assimilating activity - is used for preparation of cellulose substance by culturing.

PI JP 09131183 A 19970520 (199730)* 8p C12N015-09
JP 3077023 B2 20000814 (200043) 8p C12N015-09

L66 ANSWER 55 OF 187 WPIDS (C) 2002 THOMSON DERWENT

TI DNA for producing transgenic plants - comprises sequence encoding enzyme causing localised cell death in plants.

PI DE 19621572 A1 19971204 (199803)* 14p A01H005-00
WO 9745547 A2 19971204 (199803) DE 33p C12N015-82
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA CN IL JP US
AU 9731684 A 19980105 (199821) C12N015-82

IN MULLER-ROBER, B; WILLMITZER, L; MUELLER-ROEBER, B

L66 ANSWER 56 OF 187 WPIDS (C) 2002 THOMSON DERWENT

TI Modified fructosyl transferase gene encoding protein with inulin sucrose activity - for creating transgenic plants that produce high-molecular-weight inulin.

PI DE 19617687 A1 19971106 (199751)* 13p A01H005-00
WO 9742331 A1 19971113 (199751) DE 41p C12N015-82
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU JP US
AU 9727741 A 19971126 (199813) C12N015-82
EP 944727 A1 19990929 (199945) DE C12N015-82
R: AT BE DE DK ES FI FR GB GR IE IT LU NL SE
AU 718897 B 20000420 (200029) C12N015-82
DE 19617687 C2 20001116 (200059) A01H005-00
US 6255562 B1 20010703 (200140) C12N015-31
JP 2001517930 W 20011009 (200174) 27p C12N015-09

IN HEYER, A G; WENDENBURG, R

L66 ANSWER 57 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 22

TI Influence of intra- and extracellular sucrases of Zymomonas mobilis on the ethanol production and by-product formation.

SO Biotechnology Letters, (1997) 19/7 (661-664).

Refs: 20

ISSN: 0141-5492 CODEN: BILED3

AU Kannan T.R.; Sangiliyandi G.; Gunasekaran P.

AN 97229300 EMBASE

L66 ANSWER 58 OF 187 HCAPLUS COPYRIGHT 2002 ACS

TI Fructan synthesis in transgenic tobacco and chicory plants expressing barley sucrose:fructan 6-fructosyltransferase

SO FEBS Letters (1997), 400(3), 355-358

CODEN: FEBLAL; ISSN: 0014-5793

AU Sprenger, Norbert; Schellenbaum, Luisa; van Dun, Kees; Boller, Thomas; Wiemken, Andres

AN 1997:43935 HCAPLUS

DN 126:129269

- L66 ANSWER 59 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 23
 TI Phosphate availability in combination with nitrate availability affects root yield and chicon yield and quality of Belgian endive (*Cichorium intybus*)
 SO PLANT AND SOIL, (APR 1997) Vol. 191, No. 2, pp. 269-277.
 Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
 ISSN: 0032-079X.
 AU Ameziane R; Cassan L; Dufosse C; Rufty T W; Limami A M (Reprint)
 AN 97:687077 SCISEARCH
- L66 ANSWER 60 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 24
 TI Enhanced production of **fructosyltransferase** and glucosyltransferase by substrate-feeding cultures of *Aureobasidium pullulans*.
 SO Journal of Fermentation and Bioengineering, (1997) 84/3 (261-263).
 Refs: 18
 ISSN: 0922-338X CODEN: JFBIEX
 AU Jong Won Yun; Dong Hyun Kim; Seung Koo Song
 AN 97328535 EMBASE
- L66 ANSWER 61 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 25
 TI Identification of **products** formed by a **fructan: fructan fructosyltransferase** activity from *Lolium rigidum*
 SO NEW PHYTOLOGIST, (FEB 1997) Vol. 135, No. 2, pp. 249-257.
 Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
 ISSN: 0028-646X.
 AU StJohn J A; Sims I M; Bonnett G D; Simpson R J (Reprint)
 AN 97:284342 SCISEARCH
- L66 ANSWER 62 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Production of fructo-oligosaccharides by levansucrase from *Bacillus subtilis* C4
 SO Process Biochemistry (Oxford) (1997), 32(3), 237-243
 CODEN: PBCHE5; ISSN: 1359-5113
 AU Euzenat, O.; Guibert, A.; Combes, D.
 AN 1997:106570 HCAPLUS
 DN 126:208843
- L66 ANSWER 63 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 26
 TI Molecular characterization of natural *Erwinia amylovora* strains deficient in levan synthesis
 SO PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, (OCT 1997) Vol. 51, No. 4, pp. 215-225.
 Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON, ENGLAND NW1 7DX.
 ISSN: 0885-5765.
 AU Bereswill S; Jock S; Aldridge P; Janse J D; Geider K (Reprint)
 AN 1998:163157 SCISEARCH
- L66 ANSWER 64 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 27
 TI Biosynthesis in vitro of high-molecular-mass fructan by cell-free extracts from tuberous roots of *Viguiera discolor* (Asteraceae)
 SO NEW PHYTOLOGIST, (MAY 1997) Vol. 136, No. 1, pp. 53-60.
 Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211.
 ISSN: 0028-646X.
 AU Itaya N M; Buckeridge M S; FigueiredoRibeiro R C L (Reprint)
 AN 97:456023 SCISEARCH
- L66 ANSWER 65 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI Analysis of structure-function relationship in fructosyltransferase of *Zymomonas mobilis*.

SO Journal of Applied Glycoscience, (1997), 44 (2) 203-211, 17 ref.
ISSN: 1340-3494
AU Yanase, H.
AN 1998(05):B0630 FSTA

L66 ANSWER 66 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Production of fructo-oligosaccharides by levansucrase from *Bacillus subtilis* C4.
SO Process Biochemistry, (1997), 32 (3) 237-243, 13 ref.
ISSN: 0032-9592
AU Euzenat, O.; Guibert, A.; Combes, D.
AN 1997(12):B0032 FSTA

L66 ANSWER 67 OF 187 WPIDS (C) 2002 THOMSON DERWENT
TI Liq. food materials which retain original flavour - contain fructo-oligosaccharide(s), and is obtd. using agricultural prodn. contg. sucrose.
PI JP 08173109 A 19960709 (199637)* 15p A23L002-02
JP 2852206 B2 19990127 (199909) 15p A23L002-02

L66 ANSWER 68 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 28
TI PURIFICATION AND CHARACTERIZATION OF THE ENZYMES OF FRUCTAN BIOSYNTHESIS IN TUBERS OF *HELIANTHUS-TUBEROSUS* COLOMBIA .2. PURIFICATION OF SUCROSE-SUCROSE 1-**FRUCTOSYLTRANSFERASE** AND RECONSTITUTION OF **FRUCTAN SYNTHESIS** IN-VITRO WITH PURIFIED SUCROSE-SUCROSE 1-FRUCTOSYLTRANSFERASE AND FRUCTAN-FRUCTAN 1-FRUCTOSYLTRANSFERASE
SO PLANT PHYSIOLOGY, (APR 1996) Vol. 110, No. 4, pp. 1167-1175.
ISSN: 0032-0889.
AU KOOPS A J (Reprint); JONKER H H
AN 96:310739 SCISEARCH

L66 ANSWER 69 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Production of levan, a fructose polymer, using an overexpressed recombinant levansucrase;
Zymomonas mobilis gene expression in *Escherichia coli* (conference paper)
SO Ann.N.Y.Acad.Sci.; (1996) 799, 601-07
CODEN: ANYAA9 ISSN: 0077-8923
Enzyme Engineering XIII, San Diego, CA, 15-20 October, 1995.
AU Song K B; Belghith H; *Rhee S K
AN 1997-03371 BIOTECHDS

L66 ANSWER 70 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Synthesis of fructans in tubers of transgenic starch-deficient potato plants does not result in an increased allocation of carbohydrates
SO Planta (1996), 199(4), 528-536
CODEN: PLANAB; ISSN: 0032-0935
AU Roeber, Manuela; Geider, Klaus; Mueller-Roeber, Bernd; Willmitzer, Lothar
AN 1996:531174 HCAPLUS
DN 125:190857

L66 ANSWER 71 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)
TI **INULIN SYNTHESIS** WITH PURIFIED **FRUCTOSYL-TRANSFERASES** (SST AND FFT) FROM JERUSALEM-ARTICHOKE TUBERS (*HELIANTHUS-TUBEROSUS* L)
SO PLANT PHYSIOLOGY, (JUN 1996) Vol. 111, No. 2, Supp. S, pp. 380.
ISSN: 0032-0889.
AU LUSCHER M (Reprint); ERDIN C; SPRENGER N; HOCHSTRASSER U; WIEMKEN A
AN 96:492052 SCISEARCH

L66 ANSWER 72 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 29
TI IN-VITRO SYNTHESIS OF FRUCTOFURANOSYL-ONLY OLIGOSACCHARIDES FROM INULIN AND FRUCTOSE BY PURIFIED CHICORY ROOT FRUCTAN-FRUCTAN FRUCTOSYLTRANSFERASE

SO PHYSIOLOGIA PLANTARUM, (JUN 1996) Vol. 97, No. 2, pp. 346-352.
ISSN: 0031-9317.

AU VANDENENDE W; DEROOVER J; VANLAERE A (Reprint)
AN 96:484264 SCISEARCH

L66 ANSWER 73 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 30
TI FRUCTAN METABOLISM IN EXPANDING LEAVES, MATURE LEAF SHEATHS AND MATURE
LEAF BLADES OF LOLIUM-PERENNE - **FRUCTAN SYNTHESIS**,
FRUCTOSYLTRANSFERASE AND INVERTASE ACTIVITIES
SO NEW PHYTOLOGIST, (OCT 1996) Vol. 134, No. 2, pp. 205-214.
ISSN: 0028-646X.
AU GUERRAND D; PRUDHOMME M P (Reprint); BOUCAUD J
AN 96:818380 SCISEARCH

L66 ANSWER 74 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Hypolipidemic effect of levan
SO Mikrooekologie und Therapie (1996), 24(Proceedings of the XI.
International Symposium on Gnotobiology, 1996), 195-199
CODEN: MITHE4; ISSN: 0720-0536
AU Takahashi, Masao; Ishihara, Kazuoki
AN 1997:543893 HCAPLUS
DN 127:204922

L66 ANSWER 75 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI **Inulin synthesis** with purified **fructosyl-**
transferases (SST and FFT) from Jerusalem artichoke tubers
(Helianthus tuberosus L.
SO Plant Physiology (Rockville), (1996) Vol. 111, No. 2 SUPPL., pp. 99.
Meeting Info.: Annual Meeting of the American Society of Plant
Physiologists San Antonio, Texas, USA July 27-31, 1996
ISSN: 0032-0889.
AU Luscher, Marcel; Erdin, Christian; Sprenger, Norbert; Hochstrasser, Urs;
Wiemken, Andres
AN 1996:358153 BIOSIS

L66 ANSWER 76 OF 187 MEDLINE DUPLICATE 31
TI **Inulin synthesis** by a combination of purified
fructosyltransferases from tubers of Helianthus tuberosus.
SO FEBS LETTERS, (1996 Apr 29) 385 (1-2) 39-42.
Journal code: 0155157. ISSN: 0014-5793.
AU Luscher M; Erdin C; Sprenger N; Hochstrasser U; Boller T; Wiemken A
AN 96200165 MEDLINE

L66 ANSWER 77 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Optimal conditions for levan formation by an overexpressed recombinant
levansucrase.
SO Biotechnology Letters, (1996), 18 (4) 467-472, 15 ref.
ISSN: 0141-5492
AU Hafedh Belghith; Ki-Bang Song; Chul-Ho Kim; Sang-Ki Rhee
AN 1996(10):B0089 FSTA

L66 ANSWER 78 OF 187 FSTA COPYRIGHT 2002 IFIS
TI Purification and characterization of the enzymes of fructan biosynthesis
in tubers of Helianthus tuberosus Columbia. II. Purification of
sucrose:sucrose 1-**fructosyltransferase** and reconstitution of
fructan synthesis in vitro with purified sucrose:sucrose
1-fructosyltransferase and fructan:fructan 1-fructosyltransferase.
SO Plant Physiology, (1996), 110 (4) 1167-1175, 24 ref.
ISSN: 0032-0889
AU Koops, A. J.; Jonker, H. H.
AN 1996(09):J0133 FSTA

L66 ANSWER 79 OF 187 FSTA COPYRIGHT 2002 IFIS
TI In vitro synthesis of fructofuranosyl-only oligosaccharides from inulin

and fructose by purified chicory root fructan:fructan fructosyl transferase.

SO Physiologia Plantarum, (1996), 97 (2) 346-352, 18 ref.
ISSN: 0031-9317

AU Ende, W. van den; Roover, J. de; Laere, A. van
AN 1997(01):H0001 FSTA

L66 ANSWER 80 OF 187 FSTA COPYRIGHT 2002 IFIS
TI **Inulin synthesis** by a combination of purified
fructosyltransferases from tubers of *Helianthus tuberosus*.
SO FEBS Letters, (1996), 385 (1/2) 39-42, 21 ref.
ISSN: 0014-5793
AU Luescher, M.; Erdin, C.; Sprenger, N.; Hochstrasser, U.; Boller, T.;
Wiemken, A.
AN 1996(07):B0058 FSTA

L66 ANSWER 81 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Fructose polymers are produced by transgenic crop plants;
fructan, dextran, alternan production in maize, potato and tobacco;
DNA construct containing tissue-specific promoter, vacuole targeting
sequence and fructosyltransferase gene
AU Caimi P G; Hershey H P; Kerr P S
AN 1995-09028 BIOTECHDS
PI WO 9513389 18 May 1995

L66 ANSWER 82 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI *Bacillus licheniformis* NRRL B-18962 capable of **producing**
levan sucrase in the absence of sucrose.
SO Official Gazette of the United States Patent and Trademark Office Patents,
(Jan. 10, 1995) Vol. 1170, No. 2, pp. 1174.
ISSN: 0098-1133.
AU Charles, R. L.; Shetty, J. K.
AN 2002:13383 BIOSIS

L66 ANSWER 83 OF 187 WPIDS (C) 2002 THOMSON DERWENT
TI New fructosyl transferase from *Acetobacter diazotrophicus* - for the prodn.
of fructo-oligosaccharide and fructan cpds. from sucrose, useful e.g. as
low calorie sweeteners..
PI EP 663442 A1 19950719 (199533)* EN 16p C12N009-10
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
AU 9480431 A 19950706 (199534) C12N009-10
BR 9405235 A 19950801 (199544) C12P019-34
JP 08047394 A 19960220 (199617) 21p C12N015-09
CA 2141979 A 19960808 (199649)# C12N015-54
US 5641667 A 19970624 (199731) 11p C12N009-10
AU 682727 B 19971016 (199801) C12N009-10
US 5731173 A 19980324 (199819) 10p C12P019-18
EP 663442 B1 19990804 (199935) EN C12N009-10
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
DE 69419892 E 19990909 (199943) C12N009-10
ES 2138645 T3 20000116 (200011) C12N009-10
IN GARCIA, L H; GONZALEZ, A C; SOSA, G S; SOSA, J G A; HERNANDEZ, GARCIA L

L66 ANSWER 84 OF 187 MEDLINE DUPLICATE 32
TI Purification, cloning, and functional expression of sucrose:fructan 6-
fructosyltransferase, a key enzyme of **fructan**
synthesis in barley.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
AMERICA, (1995 Dec 5) 92 (25) 11652-6.
Journal code: 7505876. ISSN: 0027-8424.
AU Sprenger N; Bortlik K; Brandt A; Boller T; Wiemken A
AN 96102172 MEDLINE

L66 ANSWER 85 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 33

TI SUCROSE-FRUCTAN 6-FRUCTOSYLTRANSFERASE, A KEY ENZYME FOR DIVERTING CARBON
FROM SUCROSE TO FRUCTAN IN BARLEY LEAVES
SO PLANT PHYSIOLOGY, (APR 1995) Vol. 107, No. 4, pp. 1249-1255.
ISSN: 0032-0889.
AU DUCHATEAU N; BORTLIK K; SIMMEN U; WIEMKEN A; BANCAL P (Reprint)
AN 95:281527 SCISEARCH

L66 ANSWER 86 OF 187 LIFESCI COPYRIGHT 2002 CSA
TI Bacillus licheniformis NRRL B-18962 capable of **producing**
levan sucrase in the absence of sucrose
SO (1995) . US Patent 5,380,661; US Cl. 435/252.5; Int. Cl. C12N 1/20, 1/00..
AU Charles, R.L.; Shetty, J.K.
AN 95:29078 LIFESCI

L66 ANSWER 87 OF 187 LIFESCI COPYRIGHT 2002 CSA
TI Bacillus licheniformis NRRL B-18962 capable of **producing**
levan sucrase in the absence of sucrose
SO (1995) . US Patent 5380661; US Cl. 435/252.5 435/836.
AN 96:76963 LIFESCI

L66 ANSWER 88 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Method for transgenic plant construction with modified fructan content;
potato transformation with plant, Bacillus subtilis or Streptococcus
mutans **fructosyltransferase** gene for **fructan**
production and increased stress tolerance
AN 1994-11116 BIOTECHDS
PI WO 9414970 7 Jul 1994

L66 ANSWER 89 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Branched chain inulo-oligo sugar;
fructosylated branched chain cyclic inulo-oligosaccharide production
by beta-2,1-binding of **product** from **inulin**
conversion by **fructosyltransferase**
AN 1995-00585 BIOTECHDS
PI JP 06263802 20 Sep 1994

L66 ANSWER 90 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Production of fructosyl-trehalose;
using Aspergillus sydowii producing **fructosyltransferase**
AN 1995-01493 BIOTECHDS
PI JP 06284894 11 Oct 1994

L66 ANSWER 91 OF 187 MEDLINE DUPLICATE 35
TI Genetic analysis of fructan-hyperproducing strains of Streptococcus
mutans.
SO INFECTION AND IMMUNITY, (1994 Jul) 62 (7) 2679-86.
Journal code: 0246127. ISSN: 0019-9567.
AU Kiska D L; Macrina F L
AN 94274278 MEDLINE

L66 ANSWER 92 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 36
TI ENZYMATIC-**SYNTHESIS** OF **LEVAN** BY ZYMOMONAS-MOBILIS
LEVANSUCRASE OVEREXPRESSED IN ESCHERICHIA-COLI
SO BIOTECHNOLOGY LETTERS, (DEC 1994) Vol. 16, No. 12, pp. 1305-1310.
ISSN: 0141-5492.
AU SONG K B; RHEE S K (Reprint)
AN 95:37288 SCISEARCH

L66 ANSWER 93 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
TI Accumulation of fructose polymers in transgenic tobacco;
leaf disk culture transformation by Agrobacterium tumefaciens for
Bacillus subtilis **levansucrase** expression; potential
fructan production in transgenic plant
SO Bio/Technology; (1994) 12, 3, 272-75

CODEN: BTCHDA

AU Ebskamp M J M; van der Meer I M; Spronk B A; Weisbeek P J; *Smeekens S C
M

AN 1994-05107 BIOTECHDS

L66 ANSWER 94 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 37

TI BIOCHEMICAL AND ECONOMICAL ASPECTS OF LEVAN SYNTHESIS BY ZYMOMONAS-MOBILIS

SO BIOCATALYSIS, (1994) Vol. 10, No. 1-4, pp. 131-136.
ISSN: 0886-4454.

AU HARTMEIER W (Reprint); REISS M; HEIDEL M; MARX S

AN 95:314006 SCISEARCH

L66 ANSWER 95 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

TI Fructan accumulation in transgenic plants;
fructosyltransferase gene cloning and vacuole-, apoplast- and
cytosol-specific gene expression in potato and tobacco transgenic
plant (conference abstract)

SO J.Cell.Biochem.; (1994) Suppl.18A, 119
CODEN: JCEBD5

AU Ebskamp M J M; van der Meer I M; Pilon-Smits E A H; Weisbeek P J;
Smeekens S C M

AN 1994-13622 BIOTECHDS

L66 ANSWER 96 OF 187 LIFESCI COPYRIGHT 2002 CSA

TI Process for **producing levan sucrase** using
Bacillus licheniformis

SO (1994) . US Patent 5,334,524; US Cl. 435/193; Int. Cl. C12N 9/00, 1/20,
9/10; A61K 37/50..

AU Charles, R.L.; Shetty, J.K.

AN 95:40882 LIFESCI

L66 ANSWER 97 OF 187 FSTA COPYRIGHT 2002 IFIS

TI Enzymatic **synthesis** of **levan** by Zymomonas mobilis
levansucrase overexpressed in Escherichia coli.

SO Biotechnology Letters, (1994), 16 (12) 1305-1310, 21 ref.
ISSN: 0141-5492

AU Ki-Bang Song; Sang-Ki Rhee

AN 1995(04):B0108 FSTA

L66 ANSWER 98 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 38

TI FRUCTAN CONTENT OF WHEAT SEEDLINGS (TRITICUM-AESTIVUM L) UNDER HYPOXIA AND
FOLLOWING RE-AERATION

SO NEW PHYTOLOGIST, (MAR 1993) Vol. 123, No. 3, pp. 471-476.
ISSN: 0028-646X.

AU ALBRECHT G (Reprint); KAMMERER S; PRAZNIK W; WIEDENROTH E M

AN 93:276057 SCISEARCH

L66 ANSWER 99 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 39

TI SPECIES-DEPENDENT PATTERNS OF FRUCTAN SYNTHESIS BY ENZYMES FROM EXCISED
LEAVES OF OAT, WHEAT, BARLEY AND TIMOTHY

SO NEW PHYTOLOGIST, (JUL 1993) Vol. 124, No. 3, pp. 381-388.
ISSN: 0028-646X.

AU CAIRNS A J (Reprint); ASHTON J E

AN 93:498412 SCISEARCH

L66 ANSWER 100 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 40

TI **PRODUCTION** AND ISOLATION OF **LEVAN** BY USE OF
LEVANSUCRASE IMMOBILIZED ON THE CERAMIC SUPPORT SM-10

SO BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY, (FEB 1993) Vol. 57, No. 2, pp.
322-324.
ISSN: 0916-8451.

AU IIZUKA M (Reprint); YAMAGUCHI H; ONO S; MINAMIURA N

AN 93:163205 SCISEARCH

L66 ANSWER 101 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Enzymic synthesis of oligo- and polysaccharides
 SO Denpun Kagaku (1993), 40(2), 203-8
 CODEN: DPNKAV; ISSN: 0021-5406
 AU Tanaka, Toshio
 AN 1993:576807 HCAPLUS
 DN 119:176807

L66 ANSWER 102 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI [Enzymic synthesis of oligo- and polysaccharides.]
 SO Denpun Kagaku, (1993), 40 (2) 203-208, 24 ref.
 ISSN: 0021-5406
 AU Tanaka, T.
 AN 1994(01):B0088 FSTA

L66 ANSWER 103 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI **Production** and isolation of **levan** by use of
levansucrase immobilized on the ceramic support SM-10.
 SO Bioscience, Biotechnology, and Biochemistry, (1993), 57 (2) 322-324, 6
 ref.
 AU Iizuka, M.; Yamaguchi, H.; Ono, S.; Minamiura, N.
 AN 1993(08):B0111 FSTA

L66 ANSWER 104 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI New oligosaccharide sweetener and its production;
 by using *Rahnella aquatilis* **levansucrase**
 AN 1992-09317 BIOTECHDS
 PI JP 04103593 6 Apr 1992

L66 ANSWER 105 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 42
 TI **LEVAN AND LEVANSUCRASE SYNTHESIZED BY THE**
 FIREBLIGHT PATHOGEN ERWINIA-AMYLOVORA
 SO PHYSIOLOGICAL AND MOLECULAR PLANT PATHOLOGY, (JUN 1992) Vol. 40, No. 6,
 pp. 371-381.
 ISSN: 0885-5765.
 AU GROSS M; GEIER G; RUDOLPH K; GEIDER K (Reprint)
 AN 92:572890 SCISEARCH

L66 ANSWER 106 OF 187 WPIDS (C) 2002 THOMSON DERWENT
 TI Novel heat-resistant **levan-sucrase** enzyme - is
produced from aerobic cultures of *Rahnella* spp. bacteria.
 PI JP 03198773 A 19910829 (199141)*
 JP 08000071 B2 19960110 (199606) 5p C12N009-10

L66 ANSWER 107 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Fructan metabolism in leaves of *Lolium rigidum* Gaudin. II.
 Fructosyltransferase, invertase and fructan hydrolase activity
 SO New Phytologist (1991), 119(4), 517-26
 CODEN: NEPHAV; ISSN: 0028-646X
 AU Smouter, Henk; Simpson, Richard J.
 AN 1992:191135 HCAPLUS
 DN 116:191135

L66 ANSWER 108 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI **Synthesis of fructan** and oligosaccharides by microbial
fructosyltransferases
 SO Denpun Kagaku (1991), 38(2), 217-22
 CODEN: DPNKAV; ISSN: 0021-5406
 AU Iizuka, Masaru; Tanaka, Toshio; Yamamoto, Satoru; Yoneda, Yukio; Itokawa,
 Shigekazu; Hiyama, Masato; Ito, Kazuo; Furuichi, Kimiaki; Minamiura,
 Noshi; Yamamoto, Takehiko
 AN 1991:678091 HCAPLUS
 DN 115:278091

L66 ANSWER 109 OF 187 MEDLINE DUPLICATE 43
 TI Polymerase and hydrolase activities of *Bacillus subtilis* levansucrase can
 be separately modulated by site-directed mutagenesis.
 SO BIOCHEMICAL JOURNAL, (1991 Oct 1) 279 (Pt 1) 35-41.
 Journal code: 2984726R. ISSN: 0264-6021.
 AU Chambert R; Petit-Glatron M F
 AN 92028833 MEDLINE

L66 ANSWER 110 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI Polymerase and hydrolase activities of *Bacillus subtilis* levansucrase can
 be separately modulated by site-directed mutagenesis.
 SO Biochemical Journal, (1991), 279 (1) 35-41, 23 ref.
 ISSN: 0264-6021
 AU Chambert, R.; Petit-Glatron, M. F.
 AN 1992(05):B0058 FSTA

L66 ANSWER 111 OF 187 MEDLINE
 TI The influence of sucralose on bacterial metabolism.
 SO JOURNAL OF DENTAL RESEARCH, (1990 Aug) 69 (8) 1480-4.
 Journal code: 0354343. ISSN: 0022-0345.
 AU Young D A; Bowen W H
 AN 90347112 MEDLINE

L66 ANSWER 112 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)
 TI SECRETION OF LEVAN SUCRASE BY GLUCONOBACTER-OXYDANS
 SO MICROBIOLOGY, (1990) Vol. 59, No. 4, pp. 405-409.
 AU TKACHENKO A A (Reprint); SERGEEVA O V
 AN 91:277482 SCISEARCH

L66 ANSWER 113 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI Microbial levan;
 produced by *Bacillus polymyxa*; levansucrase activity on sucrose
 culture medium; a review
 SO Adv.Appl.Microbiol.; (1990) 35, 171-94
 CODEN: ADAMAP
 AU Han Y W
 AN 1991-00988 BIOTECHDS

L66 ANSWER 114 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Levan and levan sucrase from the fireblight pathogen *Erwinia amylovora*
 SO Plant Pathog. Bact., Proc. Int. Conf., 7th (1990), Meeting Date 1989,
 Volume Pt. A, 81-4. Editor(s): Klement, Z. Publisher: Akad. Kiado,
 Budapest, Hung.
 CODEN: 57JIAD
 AU Gross, M.; Geier, G.; Geider, K.; Rudolph, K.
 AN 1991:627922 HCAPLUS
 DN 115:227922

L66 ANSWER 115 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI Levan production with a flocculent strain of *Zymomonas mobilis*;
 conditions and culture medium optimization (conference paper)
 SO Food Biotechnol.; (1990) 4, 1, 69-75
 CODEN: FBIOEE
 AU Reiss M; Hartmeier W
 AN 1992-01584 BIOTECHDS

L66 ANSWER 116 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI DNA sequence containing *Bacillus subtilis* *sacU* locus;
 for inducing protein e.g. levansucrase over-production in
 microorganisms; DNA sequence; vector
 AN 1990-01150 BIOTECHDS
 PI WO 8909264 5 Oct 1989

L66 ANSWER 117 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

TI Transformed **lactic acid bacterium** produced
 by electroporation;
 producing a silage probiotic compound
 AN 1989-06482 BIOTECHDS
 PI WO 8901970 9 Mar 1989

L66 ANSWER 118 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 DUPLICATE 45
 TI LOCALIZATION OF THE ENZYMES OF FRUCTAN METABOLISM IN VACUOLES ISOLATED BY
 A MECHANICAL METHOD FROM TUBERS OF JERUSALEM ARTICHOKE
 HELIANTHUS-TUBEROSUS L.
 SO PLANT PHYSIOL (BETHESDA), (1989) 89 (2), 658-663.
 CODEN: PLPHAY. ISSN: 0032-0889.
 AU DARWEN C W E; JOHN P
 AN 1989:207394 BIOSIS

L66 ANSWER 119 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI Levan biosynthesis by *Bacillus polymyxa*;
 levansucrase activity determination
 SO Mikrobiologiya; (1989) 58, 3, 457-61
 CODEN: MIKBA5
 AU Tkachenko A A; Sevryugina T V
 AN 1989-12738 BIOTECHDS

L66 ANSWER 120 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 DUPLICATE 46
 TI FRUCTAN METABOLISM IN EXPANDED PRIMARY LEAVES OF BARLEY HORDEUM-VULGARE L.
 CULTIVAR GERBEL CHANGE UPON AGEING AND SPATIAL ORGANIZATION ALONG THE LEAF
 BLADE.
 SO J PLANT PHYSIOL, (1989) 134 (2), 237-242.
 CODEN: JPPHEY. ISSN: 0176-1617.
 AU WAGNER W; WIEMKEN A
 AN 1989:294396 BIOSIS

L66 ANSWER 121 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 47
 TI PROPERTIES OF **FRUCTOSYLTRANSFERASES** INVOLVED IN THE
SYNTHESIS OF FRUCTAN IN LILIACEOUS PLANTS
 SO JOURNAL OF PLANT PHYSIOLOGY, (1989) Vol. 134, No. 2, pp. 151-155.
 AU SHIOMI N (Reprint)
 AN 89:213331 SCISEARCH

L66 ANSWER 122 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI The **production** of fructooligosaccharides from **inulin**
 or sucrose using inulinase or **fructosyltransferase** from
Aspergillus ficuum
 SO Denpun Kagaku (1989), 36(2), 103-11
 CODEN: DPNKAV; ISSN: 0021-5406
 AU Norman, Barrie E.; Hoejer-Pedersen, Birgitte
 AN 1989:552001 HCAPLUS
 DN 111:152001

L66 ANSWER 123 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI Localization of the enzymes of fructan metabolism in vacuoles isolated by
 a mechanical method from tubers of Jerusalem artichoke (*Helianthus*
tuberosus L.).
 SO Plant Physiology, (1989), 89 (2) 658-663, 16 ref.
 ISSN: 0032-0889
 AU Darwin, C. W. E.; John, P.
 AN 1990(01):J0078 FSTA

L66 ANSWER 124 OF 187 FSTA COPYRIGHT 2002 IFIS
 TI The **production** of fructooligosaccharides from **inulin**
 or sucrose using inulinase or **fructosyltransferase**.
 SO Journal of the Japanese Society of Starch Science [Denpun Kagaku], (1989),

36 (2) 103-111, 10 ref.

ISSN: 0021-5406

AU Norman, B. E.; Hojer-Pedersen, B.

AN 1991(07):B0033 FSTA

L66 ANSWER 125 OF 187 HCAPLUS COPYRIGHT 2002 ACS

TI Detection of enzymically formed carbon-14-labelled fructan oligomers by means of size exclusion chromatography with an on-line liquid scintillation counter

SO Chromatographia (1988), 26, 359-62

CODEN: CHRGB7; ISSN: 0009-5893

AU Praznik, W.; Beck, R. H. F.; Spies, T.; Huber, A.

AN 1990:213245 HCAPLUS

DN 112:213245

L66 ANSWER 126 OF 187 HCAPLUS COPYRIGHT 2002 ACS

TI Lactose-based oligosaccharides

SO Kem. - Kemi (1988), 15(3), 218-22

CODEN: KMKMAA; ISSN: 0355-1628

AU Hartikainen, Marianne; Harju, Matti; Heikonen, Matti; Linko, Pekka

AN 1988:471892 HCAPLUS

DN 109:71892

L66 ANSWER 127 OF 187 WPIDS (C) 2002 THOMSON DERWENT

TI **Lactobacillus** bifidus increase accelerators, useful for foods - contg. saccharide(s), aldo-pyranose or sugar alcohol bonded with 1-3 molecules of fructose.

PI JP 62207286 A 19870911 (198742)* 10p

L66 ANSWER 128 OF 187 MEDLINE

DUPLICATE 48

TI Isolation of DNA encoding sucrase genes from Streptococcus salivarius and partial characterization of the enzymes expressed in Escherichia coli.

SO JOURNAL OF BACTERIOLOGY, (1987 Aug) 169 (8) 3679-84.

Journal code: 2985120R. ISSN: 0021-9193.

AU Houck C M; Pear J R; Elliott R; Perchorowicz J T

AN 87279919 MEDLINE

L66 ANSWER 129 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

DUPLICATE 49

TI ENZYMOLOGY OF FRUCTAN SYNTHESIS IN GRASSES PROPERTIES OF SUCROSE-SUCROSE-FRUCTOSYLTRANSFERASE IN BARLEY LEAVES HORDEUM-VULGARE L. CULTIVAR GERBEL.

SO PLANT PHYSIOL (BETHESDA), (1987) 85 (3), 706-710.

CODEN: PLPHAY. ISSN: 0032-0889.

AU WAGNER W; WIEMKEN A

AN 1988:94703 BIOSIS

L66 ANSWER 130 OF 187 MEDLINE

DUPLICATE 50

TI Metabolism of glycosylsucrose by oral microorganisms and its hydrolysis by Streptococcus salivarius fructosyltransferase.

SO INFECTION AND IMMUNITY, (1987 Mar) 55 (3) 698-703.

Journal code: 0246127. ISSN: 0019-9567.

AU Hojo S; Mitsutomi M; Yamada T

AN 87136190 MEDLINE

L66 ANSWER 131 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

TI **FRUCTAN SYNTHESIS** IN BARLEY LEAVES SUCROSE SUCROSE **FRUCTOSYLTRANSFERASE** SST.

SO ANNUAL MEETING OF THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ST. LOUIS, MISSOURI, USA, JULY 19-23, 1987. PLANT PHYSIOL (BETHESDA). (1987) 83 (4 SUPPL), 69.

CODEN: PLPHAY. ISSN: 0032-0889.

AU WAGNER W; WIEMKEN A

AN 1987:373342 BIOSIS

L66 ANSWER 132 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI Use of transferases in enzymatic synthesis reactions;
 using glycosyltransferase and phosphoryltransferase reactions
 (conference paper)
 SO World Biotech Rep.; (1987) 1, Pt.4, 53-59
 AU Monsan P; Paul F
 AN 1988-03199 BIOTECHDS

L66 ANSWER 133 OF 187 WPIDS (C) 2002 THOMSON DERWENT
 TI Culture liquor contg. fructo-oligosaccharide and beta-1,3-1,6-glucane -
 prepd. by culturing yeast of aureobasidium in liq. culture medium contg.
 saccharose rice bran vitamin(s) C and E.
 PI JP 61146192 A 19860703 (198633)* 8p
 JP 05004063 B 19930119 (199306) 9p A23L002-00

L66 ANSWER 134 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Fructans and the metabolism of sucrose in vascular plants
 SO New Phytol. (1986), 104(1), 1-24
 CODEN: NEPHAV; ISSN: 0028-646X
 AU Pollock, C. J.
 AN 1986:587555 HCAPLUS
 DN 105:187555

L66 ANSWER 135 OF 187 LIFESCI COPYRIGHT 2002 CSA DUPLICATE 51
 TI Selectivity and efficiency of utilization of galactosyl-oligosaccharides
 by bifidobacteria.
 SO CHEM. PHARM. BULL. (TOKYO)., (1985) vol. 33, no. 2, pp. 710-714.
 AU Minami, Y.; Yazawa, K.; Nakamura, K.; Tamura, Z.
 AN 85:10500 LIFESCI

L66 ANSWER 136 OF 187 LIFESCI COPYRIGHT 2002 CSA
 TI **Synthesis** of **levan** catalyzed by **levan**
sucrase from *Gluconobacter oxydans* .
 SO APPL. BIOCHEM. MICROBIOL., (1985) vol. 20, no. 6, pp. 635-641.
 AU Elisashvili, V.I.
 AN 85:10520 LIFESCI

L66 ANSWER 137 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 52
 TI THE MODE OF **SYNTHESIS** OF **LEVAN** BY *BACILLUS-SUBTILIS*
LEVANSUCRASE
 SO AGRICULTURAL AND BIOLOGICAL CHEMISTRY, (1985) Vol. 49, No. 2, pp. 343-349.
 AU YAMAMOTO S (Reprint); IIZUKA M; TANAKA T; YAMAMOTO T
 AN 85:140213 SCISEARCH

L66 ANSWER 138 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Effects of sodium and potassium ions on the characteristics of oral
 bacteria
 SO Recent Adv. Streptococci Streptococcal Dis., Proc. Lancefield Int. Symp.
 Streptococci Streptococcal Dis., 9th (1985), Meeting Date 1984, 105-6.
 Editor(s): Kimura, Yoshitami; Kotani, Shozo; Shiokawa, Yuichi. Publisher:
 Reedbooks, Bracknell, UK.
 CODEN: 55BSAN
 AU Knox, K. W.; Forester, H.; Jacques, N. A.; Wicken, A. J.; Fitzgerald, R.
 J.
 AN 1986:438870 HCAPLUS
 DN 105:38870

L66 ANSWER 139 OF 187 LIFESCI COPYRIGHT 2002 CSA DUPLICATE 53
 TI **Levan synthesis**, catalysed by **levansucrase**
 from *Gluconobacter oxydans* .
 SO PRIKL. BIOKHIM. MIKROBIOL., (1984) vol. 20, no. 6, pp. 773-780.
 AU Elisashvili, V.I.
 AN 84:61551 LIFESCI

L66 ANSWER 140 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI Production purification and immobilization of *Bacillus subtilis*
 levan-sucrase;
 (conference paper)
 SO Ann.N.Y.Acad.Sci.; (1984) 434, 468-71
 CODEN: ANYAA9
 AU Perlot P; Monsan P
 AN 1985-09634 BIOTECHDS

L66 ANSWER 141 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 DUPLICATE 55
 TI LOCALIZATION OF FRUCTAN METABOLISM IN THE VACUOLES ISOLATED FROM
 PROTOPLASTS OF JERUSALEM-ARTICHOKE TUBERS *HELIANTHUS-TUBEROSUS*.
 SO J PLANT PHYSIOL, (1984) 116 (3), 197-208.
 CODEN: JPPHEY.
 AU FREHNER M; KELLER F; WIEMKEN A
 AN 1985:276707 BIOSIS

L66 ANSWER 142 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI On the synthesis of levan by the culture *Bacillus* sp.;
 optimization of levan production
 SO Prikl.Biokhim.Mikrobiol.; (1984) 20, 1, 101-06
 CODEN: PBMIK
 AU Elisashvili V I
 AN 1984-07857 BIOTECHDS

L66 ANSWER 143 OF 187 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
 TI A compound which accelerates the growth of *Lactobacillus*
 bifidus;
 preparation from sucrose and fructose using **fructosyl-**
transferase; use in treatment of intestinal disorder in human
 baby
 AN 1984-02631 BIOTECHDS
 PI JP 58201980 25 Nov 1983

L66 ANSWER 144 OF 187 LIFESCI COPYRIGHT 2002 CSA DUPLICATE 57
 TI Selectivity of utilization of galactosyl-oligosaccharides by
 Bifidobacteria.
 SO CHEM. PHARM. BULL. (TOKYO)., (1983) vol. 31, no. 5, pp. 1688-1691.
 AU Minami, Y.; Yazawa, K.; Tamura, Z.; Tanaka, T.; Yamamoto, T.
 AN 83:56498 LIFESCI

L66 ANSWER 145 OF 187 WPIDS (C) 2002 THOMSON DERWENT
 TI Accelerating agent for multiplication of bifido bacteria - contains levan
 of specified fructose polymerisation degree as effective component.
 PI JP 57018982 A 19820130 (198210)* 3p
 JP 63016118 B 19880407 (198818)

L66 ANSWER 146 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI REGULATION OF GLYCOSYL TRANSFERASES IN *STREPTOCOCCUS-SANGUIS*.
 SO 30TH ANNUAL MEETING OF THE BRITISH DIVISION OF THE INTERNATIONAL
 ASSOCIATION FOR DENTAL RESEARCH, EDINBURGH, SCOTLAND, MARCH 31-APRIL 2,
 1982. J DENT RES. (1982) 61 (4), 542.
 CODEN: JDREAF. ISSN: 0022-0345.
 AU KEEVIL C W; BOURNE N; MARSH P D; ELLWOOD D C
 AN 1983:110966 BIOSIS

L66 ANSWER 147 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Hydrolysis of glycosylsucrose by fructosyltransferase from oral bacteria
 SO Eiyo to Shokuryo (1982), 35(3), 197-200
 CODEN: EISOAU; ISSN: 0021-5376
 AU Hojo, Sachiko
 AN 1982:595516 HCAPLUS

DN 97:195516

L66 ANSWER 148 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI SERINE PROTEASE SUBTILISIN STIMULATES **LEVAN SUCRASE**
SYNTHESIS IN BACILLUS-SUBTILIS.
SO FEMS Microbiol. Lett., (1982) 13 (2), 191-194.
CODEN: FMLED7. ISSN: 0378-1097.
AU STEINMETZ M
AN 1982:138544 BIOSIS

L66 ANSWER 149 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI STRUCTURES OF HETERO OLIGO SACCHARIDES **SYNTHESIZED** BY
LEVAN SUCRASE.
SO J BIOCHEM (TOKYO), (1981) 90 (2), 521-526.
CODEN: JOBIAO. ISSN: 0021-924X.
AU TANAKA T; YAMAMOTO S; OI S; YAMAMOTO T
AN 1982:196636 BIOSIS

L66 ANSWER 150 OF 187 WPIDS (C) 2002 THOMSON DERWENT
TI Inhibition of dextran sucrase with fluoro-substd. sucrose - used in oral
compsns. to inhibit dental plaque.
PI US 4228150 A 19801014 (198044)*
IN ROBYT, J F; ZIKOPOULOS, J N

L66 ANSWER 151 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI SUCROSE SUCROSE FRUCTOSYL TRANSFERASE AND FRUCTAN FRUCTAN FRUCTOSYL
TRANSFERASE FROM ALLIUM-CEPA.
SO PHYTOCHEMISTRY (OXF), (1980) 19 (6), 1017-1020.
CODEN: PYTCAS. ISSN: 0031-9422.
AU HENRY R J; DARBYSHIRE B
AN 1980:281670 BIOSIS

L66 ANSWER 152 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI ROLE OF COMPONENTS OF GROWTH MEDIA IN REGULATING THE **SYNTHESIS**
OF **LEVAN SUCRASE** BY GLUCONOBACTER-OXYDANS.
SO MIKROBIOLOGIYA, (1980) 49 (3), 412-416.
CODEN: MIKBA5. ISSN: 0026-3656.
AU TKACHENKO A A; LOITSYANSKAYA M S; YAREMENKO T G
AN 1981:295284 BIOSIS

L66 ANSWER 153 OF 187 MEDLINE DUPLICATE 58
TI The molecular structure of low and high molecular weight levans
synthesized by levansucrase.
SO JOURNAL OF BIOCHEMISTRY, (1980 Jan) 87 (1) 297-303.
Journal code: 0376600. ISSN: 0021-924X.
AU Tanaka T; Oi S; Yamamoto T
AN 80137369 MEDLINE

L66 ANSWER 154 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
TI ROLE OF NUTRIENT MEDIUM COMPONENTS IN THE REGULATION OF **LEVAN**
SUCRASE SYNTHESIS IN GLUCONOBACTER-OXYDANS.
SO Microbiology (Engl. Transl.), (1980 (RECD 1981)) 49 (3), 289-292.
CODEN: MIBLAA. ISSN: 0026-2617.
AU TKACHENKO A A; LOITSYANSKAYA M S; YAREMENKO T G
AN 1981:73608 BIOSIS

L66 ANSWER 155 OF 187 MEDLINE DUPLICATE 59
TI **Synthesis** of **levan** by **levansucrase**. Some
factors affecting the rate of synthesis and degree of polymerization of
levan.
SO JOURNAL OF BIOCHEMISTRY, (1979 Jan) 85 (1) 287-93.
Journal code: 0376600. ISSN: 0021-924X.
AU Tanaka T; Oi S; Yamamoto T
AN 79109532 MEDLINE

L66 ANSWER 156 OF 187 MEDLINE DUPLICATE 60
 TI The cell wall-associated levansucrase of *Actinomyces viscosus*.
 SO BIOCHIMICA ET BIOPHYSICA ACTA, (1979 Feb 9) 566 (2) 274-82.
 Journal code: 0217513. ISSN: 0006-3002.
 AU Pabst M J; Cisar J O; Trummel C L
 AN 79124819 MEDLINE

L66 ANSWER 157 OF 187 MEDLINE DUPLICATE 61
 TI Levan and levansucrase of *Actinomyces viscosus*.
 SO INFECTION AND IMMUNITY, (1977 Feb) 15 (2) 518-26.
 Journal code: 0246127. ISSN: 0019-9567.
 Report No.: NASA-77140067.
 AU Pabst M J
 AN 77140067 MEDLINE

L66 ANSWER 158 OF 187 MEDLINE
 TI Sucrolytic enzymes from human dental plaque in saliva.
 SO SCANDINAVIAN JOURNAL OF DENTAL RESEARCH, (1977 Jan-Feb) 85 (2) 101-5.
 Journal code: 0270023. ISSN: 0029-845X.
 AU Aksnes A
 AN 77127127 MEDLINE

L66 ANSWER 159 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI COLONIZATION MEDIATED BY EXTRACELLULAR GLUCOSYL TRANSFERASE FROM
 STREPTOCOCCUS-SALIVARIUS.
 SO Abstr. Annu. Meet. Am. Soc. Microbiol., (1977) 77, 79.
 CODEN: ASMACK. ISSN: 0094-8519.
 AU MCCABE R M; DONKERSLOOT J A
 AN 1977:48631 BIOSIS

L66 ANSWER 160 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 DUPLICATE 62
 TI EFFECT OF CULTIVATION CONDITIONS AND INOCULUM DOSAGE ON LEVAN SYNTHESIS BY
 GLUCONOBACTER-OXYDANS L-1.
 SO PRIKL BIOKHM MIKROBIOL, (1976 (RECD 1977)) 12 (6), 876-881.
 CODEN: PBMIK. ISSN: 0555-1099.
 AU LOITSYANSKAYA M S; TKACHENKO A A
 AN 1977:198018 BIOSIS

L66 ANSWER 161 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
 TI Effects of sodium trimetaphosphate supplementation of a high sucrose diet
 on the microbial and biochemical composition of four day plaque and on
 urine calcium and phosphorus levels.
 SO Journal of Dental Research, (1976) 55/5 (787-796).
 CODEN: JDREAF
 AU Dennis D.A.; Gawronski T.H.; Cressey D.E.; Folke L.E.A.
 AN 77173020 EMBASE

L66 ANSWER 162 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI PURIFICATION AND PROPERTIES OF A CELL ASSOCIATED LEVAN SUCRASE FROM
 STREPTOCOCCUS-SALIVARIUS SS-2.
 SO ARCH ORAL BIOL, (1976) 21 (10), 565-570.
 CODEN: AOBIA. ISSN: 0003-9969.
 AU WHITAKER E J; EDWARDS J R
 AN 1977:146295 BIOSIS

L66 ANSWER 163 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI EFFECT OF GLUCOSE ON **SYNTHESIS OF LEVAN**
SUCRASE BY GLUCONOBACTER-OXYDANS.
 SO MIKROBIOLOGIYA, (1976) 45 (3), 450-454.
 CODEN: MIKBA5. ISSN: 0026-3656.
 AU TKACHENKO A A; LOITSYANSKAYA M S
 AN 1977:169770 BIOSIS

L66 ANSWER 164 OF 187 SCISEARCH COPYRIGHT 2002 ISI (R)
 TI EFFECT OF GLUCOSE ON **LEVAN SUCRASE SYNTHESIS**
 BY GLUCONOBACTER-OXYDANS
 SO MICROBIOLOGY, (1976) Vol. 45, No. 3, pp. 387-391.
 AU TKACHENKO A A (Reprint); LOITSYANSKAYA M S
 AN 77:39995 SCISEARCH

L66 ANSWER 165 OF 187 MEDLINE DUPLICATE 63
 TI [Raffinose metabolism in Gluconobacter oxydans].
 Metabolizm raffinozy u Gluconobacter oxydans.
 SO MIKROBIOLOGIYA, (1976 Mar-Apr) 45 (2) 229-33.
 Journal code: 0376652. ISSN: 0026-3656.
 AU Loitsyanskaia M S; Uspenskaia S N
 AN 76219543 MEDLINE

L66 ANSWER 166 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Metabolism of raffinose in Gluconobacter oxydans
 SO Mikrobiologiya (1976), 45(2), 229-33
 CODEN: MIKBA5
 AU Loitsyanskaya, M. S.; Uspenskaya, S. N.
 AN 1976:430462 HCAPLUS
 DN 85:30462

L66 ANSWER 167 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
 TI Variations in microbial and biochemical components of four day plaque
 during a four week controlled diet period.
 SO Journal of Dental Research, (1975) 54/4 (716-722).
 CODEN: JDREAF
 AU Dennis D.A.; Gawronski T.H.; Sudo S.Z.; et al.
 AN 76147248 EMBASE

L66 ANSWER 168 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Initial products in utilization of sucrose by Actinomyces viscosus
 SO Biken J. (1975), 18(4), 271-4
 CODEN: BKNJA5
 AU Suginaka, Hidekazu; Wada, Hajime; Kotani, Shozo
 AN 1976:132395 HCAPLUS
 DN 84:132395

L66 ANSWER 169 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V. DUPLICATE 64
 TI Use of specifically labelled sucrose for the quantitative assay of
 levansucrase activity.
 SO Archives of Oral Biology, (1974) 19/12 (1237-1238).
 CODEN: AOBIAR
 AU Whitaker E.J.; Edwards J.R.
 AN 75180781 EMBASE

L66 ANSWER 170 OF 187 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
 TI Translucent colony form of the gram negative, levan producing bacterium,
 Aerobacter levanicum.
 SO Journal of Bacteriology, (1973) 116/1 (503-506).
 CODEN: JOBAAY
 AU Takeshita M.
 AN 74093061 EMBASE

L66 ANSWER 171 OF 187 MEDLINE DUPLICATE 65
 TI **Synthesis** of a broth **levan** by a cell-bound
levansucrase from Streptococcus salivarius (SS2).
 SO ARCHIVES OF ORAL BIOLOGY, (1973 Feb) 18 (2) 239-51.
 Journal code: 0116711. ISSN: 0003-9969.
 AU Garszczyński S M; Edwards J R
 AN 73215230 MEDLINE

L66 ANSWER 172 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI **SYNTHESIS** OF A **LEVAN** BY A CELL BOUND **LEVAN**
SUCRASE FROM STREPTOCOCCUS-SALIVARIUS.
 SO Abstr. Annu. Meet. Am. Soc. Microbiol., (1972) 72, 183.
 CODEN: ASMACK. ISSN: 0094-8519.
 AU GARSZCZYNSKI S M; EDWARDS J R
 AN 1973:31229 BIOSIS

L66 ANSWER 173 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Levan sucrase of acetic acid bacteria
 SO Mikrobiologiya (1971), 40(3), 505-8
 CODEN: MIKBA5
 AU Loitsyanskaya, M. S.; Tkachenko, A. A.; Elisashvili, T. A.
 AN 1971:485541 HCAPLUS
 DN 75:85541

L66 ANSWER 174 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Levansucrase from Streptococcus mutans
 SO Caries Res. (1970), 4(2), 97-113
 CODEN: CAREBK
 AU Carlsson, Jan
 AN 1970:410792 HCAPLUS
 DN 73:10792

L66 ANSWER 175 OF 187 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
 TI GENETIC LOCALIZATIONS OF MUTANTS OF THE METABOLIC SYSTEM OF SUCROSE IN
 BACILLUS-SUBTILIS LOCALIZATION BY TRANSDUCTION WITH THE AID OF PHAGE
 PBS-1.
 SO C R HEBD SEANCES ACAD SCI SER D SCI NATUR (PARIS), (1969) 269 (17),
 1712-1715.
 CODEN: CHDDAT. ISSN: 0567-655X.
 AU LEPESANT J-A; KUNST F; CARAYON A; DEDONDER R
 AN 1970:140556 BIOSIS

L66 ANSWER 176 OF 187 MEDLINE
 TI A preliminary study of the incorporation of glucose-C14 in the enzymatic
synthesis of sucrose and **levan** by the
levansucrase system of Streptococcus salivarius.
 SO JOURNAL OF THE BALTIMORE COLLEGE OF DENTAL SURGERY, (1969 Jul) 24 (1)
 27-36.
 Journal code: 7505588. ISSN: 0067-3072.
 AU Paulis C L; Barry S C
 AN 70062100 MEDLINE

L66 ANSWER 177 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Purification of levansucrase by precipitation with levan
 SO Biochim. Biophys. Acta (1966), 113(1), 79-83
 AU Reese, Elwyn T.; Avigad, Gad
 AN 1966:45443 HCAPLUS
 DN 64:45443
 OREF 64:8557d-e

L66 ANSWER 178 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI **Synthesis** of **levan sucrase** in Bacillus
 subtilis in the presence of structural amino acid analogs
 SO Biochim. Biophys. Acta (1964), 89(2), 354-6
 AU Rapoport, G.; Dedonder, R.
 AN 1964:470827 HCAPLUS
 DN 61:70827
 OREF 61:12352f-g

L66 ANSWER 179 OF 187 HCAPLUS COPYRIGHT 2002 ACS
 TI Oscar Guire Levan sucrase in soils
 SO Naturwissenschaften (1961), 48, 700

AU St. Kiss
AN 1962:48035 HCAPLUS
DN 56:48035
OREF 56:9144f-g

L66 ANSWER 180 OF 187 MEDLINE
TI [Studies on the **levansucrase** of *Bacillus subtilis*. I.
Production of levans and **levansucrase** (**levan**
-succharotransfructosidase) by cultures of *Bacillus subtilis*].
Etudes sur la levane-sucrase de *Bacillus subtilis*. I. Production de
levanes et de levane-sucrase (levane-saccharotransfructosidase) par des
cultures de *Bacillus subtilis*.
SO Bull. Soc. chim. biol., Par, (1957) 39 (5-6) 483-97.
AU DEDONDER R; PEAUD-LENOEL C
AN 58003230 MEDLINE

L66 ANSWER 181 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI The **levan-sucrase** of *Bacillus subtilis*. I.
Production of levans and **levan-sucrase** (**levan**-
saccharotransfructosidase) by cultures of *B. subtilis*
SO Bull. soc. chim. biol. (1957), 39, 483-97
AU Dedonder, R.; Peaud-Lenoel, C.
AN 1957:77455 HCAPLUS
DN 51:77455
OREF 51:13993i

L66 ANSWER 182 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Synthesis of levan by pseudomonads
SO Nature (1956), 178, 921
AU Fuchs, A.
AN 1957:17887 HCAPLUS
DN 51:17887
OREF 51:3742d-e

L66 ANSWER 183 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Mechanism of action of **levan sucrase** of *Bacillus*
subtilis. II. **Synthesis** of diholosides analogous to saccharose
SO Compt. rend. (1955), 241, 1518-21
AU Peaud-Lenoel, Claude
AN 1956:36488 HCAPLUS
DN 50:36488
OREF 50:7193b-d

L66 ANSWER 184 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Influence of glucose on the **synthesis** of **levan** and
levan-sucrase by the cultures of *Bacillus subtilis*
SO Compt. rend. (1954), 239, 325-7
AU Dedonder, Raymond; Peaud-Lenoel, Claude
AN 1955:39846 HCAPLUS
DN 49:39846
OREF 49:7645h

L66 ANSWER 185 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI Polysaccharide production from sucrose. II
SO Biochem J. (1945), 39, 167-72
AU Avineri-Shapiro, S.; Hestrin, S.
AN 1945:32294 HCAPLUS
DN 39:32294
OREF 39:5262i,5263a

L66 ANSWER 186 OF 187 HCAPLUS COPYRIGHT 2002 ACS
TI The enzymic production of levan
SO Biochem. J. (1943), 37, 450-6
AU Hestrin, S.; Avineri-Shapiro, S.; Aschner, M.

AN 1944:6681 HCAPLUS
DN 38:6681
OREF 38:984c-f

L66 ANSWER 187 OF 187 WPIDS (C) 2002 THOMSON DERWENT
TI Levans brit 837993 provided.
PI NL 222610 B (196800)*

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

328.42

328.63

STN INTERNATIONAL LOGOFF AT 16:21:52 ON 09 OCT 2002

US 099955870FP1



Creation date: 11-10-2003

Indexing Officer: DNGUYEN8 - DANH NGUYEN

Team: OIPEBackFileIndexing

Dossier: 09995587

Legal Date: 06-19-2003

| No. | Doccode | Number of pages |
|-----|---------|-----------------|
| 1 | SRNT | 60 |

Total number of pages: 60

Remarks:

Order of re-scan issued on